

CHEMICAL INTERACTIONS AMONG MILKWEED PLANTS
(ASCLEPIADACEAE) AND LEPIDOPTERAN HERBIVORES

By

JAMES A. COHEN

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By

James A. Cohen

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Milkweed plants are widely believed to be chemically defended against insect herbivory by steroidal compounds known as cardenolides (cardiac glycosides). Moreover, it has frequently been claimed that milkweed-specializing insects have coevolved with their hostplants by breaching a cardenolide-barrier over the course of evolutionary time. However, there has been, until now, no evidence that ingested cardenolides have any defensive properties against any invertebrate herbivore. Thus, cardenolide-based models of coevolution among milkweeds and insects have had little foundation. This study seeks to provide such a foundation by comparing the ecological and physiological effects of cardenolides upon lepidopteran species which do or do not feed on milkweeds.

In accordance with evolutionary predictions, the results provide little evidence that milkweed-specializing insects pay metabolic costs of handling cardenolides. These plant chemicals appear to influence neither oviposition nor larval development in wild monarch butterflies (Danaus plexippus; Danaidae), nor to affect (even when in abnormally high concentrations) consumption, growth, or food conversion efficiency of fourth instar monarchs in the laboratory.

In contrast, the polyphagous fall armyworms (Spodoptera frugiperda; Noctuidae) exhibited reduced growth, food consumption, and food conversion efficiency in the fifth instar, even when fed moderate, natural cardenolide concentrations. Although no such negative effects were found for fifth instar velvetbean caterpillars (Anticarsia gemmatilis; Noctuidae), a species that specializes on cardenolide-free plants (i.e., legumes), younger instars of this species were severely affected by cardenolides. This serves as a warning to other researchers who frequently test only older instars and may thereby generate misleading results.

This study is the first to demonstrate that ingested cardenolides may provide a chemical barrier to herbivory by certain insects. However, a detailed review of the literature provides little support for frequent contentions that milkweed-specialists were once deterred by, and subsequently breached, such a barrier. An alternative coevolutionary model is considered which predicts (inter alia) that

certain danaid and ithomiid butterflies should utilize cardenolides as hostplant recognition cues. Although my data do not confirm this prediction for monarch butterflies, this latter model nevertheless holds considerable promise as a prime example of plant/herbivore coevolution.

CHAPTER I

INTRODUCTION

A major thrust of modern ecology involves the study of relationships between heterotrophic organisms and their food supplies. Such interactions (e.g., predator vs. prey, host vs. parasite) occur not only in ecological time but also in evolutionary time (e.g., Dawkins and Krebs, 1979; Thompson, 1982). Natural selection may be presumed to act within populations to optimize simultaneously the ability to acquire resources (e.g., Pyke et al., 1977; Krebs, 1978) and the ability to avoid becoming the acquired resources of similarly selected organisms of higher trophic levels (e.g., Edmunds, 1974; Rhoades, 1979). Clearly, then, a major component of ecological research must concern the study of the varied defenses utilized by organisms to avoid being attacked by their enemies.

In this context, plants may be viewed as subject to the attacks of both predators and parasites. Predation occurs when the plant is killed outright by another organism(s) and parasitism occurs when, as in most cases of herbivory, some of the plant's biomass is consumed, lowering plant fitness, but the plant does not die in the process

(Gilbert, 1979; Price, 1980). The theory of plant/herbivore coevolution (Ehrlich and Raven, 1964) has provided a theoretical framework for the organization of a large and diverse body of literature concerning the ability of plants to avoid enemy-attack, as well as the counter-adaptations of attacking species. In essence, plants have evolved a variety of defensive adaptations (e.g., morphological, phenological, and chemical traits) against predators and parasites. In turn, such enemies have evolved means to circumvent or counteract these defenses, leading to further elaboration of plant defensive strategies, subsequent enemy offenses, and so on.

A key system, widely regarded as providing the classic, textbook example of plant/herbivore coevolution, is that of milkweed plants (Asclepiadaceae) and their specialized herbivores, most notoriously the monarch butterfly (Danaus plexippus; Danaidae). Consider the following excerpts from Harborne's (1983) text, Ecological Biochemistry (emphases added):

What is now the classic example of plant-animal coevolution in which secondary compounds have a key role is the interaction between milkweeds, monarch butterflies, and blue jays. [p. 87]

The milkweed produces several cardiac glycosides within its tissues as a passive defense against insect feeding. [p.87]

The monarch butterfly caterpillar learns to adapt to these toxins. [p. 88]

production of the toxins continues to provide the plant with protection not only from most insects but also from all grazing animals. [p. 89]

The present dissertation is concerned precisely with this interaction, for several aspects of Harborne's summary have never been substantiated by data, despite their general acceptance among evolutionary biologists. In particular, I address the question of whether the cardiac glycosides (cardenolides) present in certain milkweed plants may, in fact, offer protection against generalist-feeding insects, but not against purportedly coevolved specialists such as monarchs. The crucial question is: Do generalists pay "costs" of ingesting cardenolides that specialists do not pay? This is the first, basic step that must be established before one can entertain claims that milkweed-specialists have breached a cardenolide-barrier during the course of coevolution.

In the remainder of this chapter, I shall briefly outline the theory of plant/herbivore coevolution and the related question of chemical defense in plants. As it is supposed that a breaching of such defenses leads to the evolution of feeding specialization (e.g., Ehrlich and Raven, 1964; Feeny, 1975), I will then contrast the two strategies of specialization and generalization, with emphasis on biochemical (detoxification and tolerance) differences. By way of introduction to the specific subject matter of this dissertation, I shall then review the chemical defense of milkweed plants in particular, focusing on cardenolides, and finally summarize what is currently known about the metabolic costs, to specialists or generalists, of ingesting these purportedly defensive chemicals.

Plant/Herbivore Coevolution

Ehrlich and Raven's (1964) formulation of plant/herbivore coevolution was derived primarily from considerations of larval feeding specialization in the Lepidoptera, the most thoroughly studied order of insect herbivores. These authors summarized the large body of observations showing that certain lepidopteran taxa (frequently subfamilies or tribes) were restricted to feeding upon specific plant taxa, and suggested that these patterns of relationships were most easily interpreted in terms of the allelochemistry of the plants. Allelochemicals are plant compounds apparently not directly required for the primary metabolic functions of the plant but having a variety of inhibitory or (in some cases) stimulatory effects upon potential or actual herbivores, pathogens, or other plants (Fraenkel, 1959, 1969; Whittaker and Feeny, 1971; Levin, 1976). In many cases the compounds have potentially toxic or digestibility-reducing effects and are presumed to have evolved in response to herbivore pressure. Ehrlich and Raven (1964) suggested that certain plants, through mutation and recombination, have elaborated novel chemicals which reduce plant palatability or suitability as food for herbivores. Thus freed from previous herbivore pressure, these plants entered a new "adaptive zone" wherein phylogenetic radiation was possible. In the course of evolution, certain herbivore groups, also through a fortuitous genetic event, breached the plant's chemical defense and, in so doing, were also able to enter (and radiate within) a new adaptive zone. Ehrlich

and Raven (1964:602) further suggested that the ability of an herbivore to tolerate one kind of defensive chemistry reduces its ability to tolerate other kinds of chemical defense. Thus, the breaching of a chemical barrier is presumably followed by the rapid evolution of herbivore feeding specialization for plants having similar defensive chemistry (commonly confamilials). This hypothesis is supported by the fact that when, in exceptional cases, oligophagous lepidopterans feed on taxonomically disparate plant species, these are frequently shown to share a defensive chemistry. In such cases the herbivore may have evolved the ability to utilize the allelochemic for hostplant recognition (Fraenkel, 1959; Smith, 1966; Rees, 1969; van Emden, 1972; Nielsen, 1978).

Extending this general coevolutionary scenario, Brower and Brower (1964) demonstrated that many of the lepidopteran families feeding on toxic plant families are themselves unpalatable and aposematically colored, while those feeding on relatively innocuous plants were typically cryptic and palatable. This suggested that, following the breaching of a plant's chemical defense, certain insects might not only be able to tolerate the allelochemicals but might store them in their tissues for their own defensive purposes. These compounds may even be incorporated into the tissues of some invertebrate predators feeding upon allelochemic-storing herbivores (e.g., Malcolm, 1981). Thus, defensive chemicals may pass through a food web, having ramifications at several trophic levels.

Berenbaum (1983) has provided an example in which each step of the evolutionary sequence postulated by Ehrlich and Raven (1964) can be supported by either direct or circumstantial evidence. She makes the following argument. (1) The production of phototoxic furanocoumarins (the toxicity of which is induced only in the presence of ultraviolet light) in numerous plant families could plausibly have evolved as a fortuitous elaboration of pre-existing biosynthetic pathways (e.g., lignin synthesis). (2) These compounds are more toxic to certain herbivorous insects than are their precursors. (3) Within the Umbelliferae, the one subfamily containing coumarins is far more diverse in number of species than are two subfamilies that lack coumarins. This is circumstantial evidence for an evolutionary radiation following the evolution of the chemical defense. (4) Various biochemical and behavioral means of circumventing the plant's defense have evolved among insects. Most striking among these is the habit of leaf-rolling among the Oecophoridae, lepidopterans that feed exclusively on Umbelliferae. While feeding within rolled leaves, the larva is shielded from ultraviolet light and consequent phototoxic effects. (5) Genera of oecophorids and papilionids which are associated with furanocoumarin-containing plants have diversified (in species numbers) more extensively than have those which feed on furanocoumarin-free hostplants. This is circumstantial evidence for an evolutionary radiation following the breaching of the plant's chemical defense.

Berenbaum's (1983) work provides one of the best-developed examples of what has been called "diffuse coevolution" (Gilbert, 1975; Fox, 1981; Futuyma and Slatkin, 1983), i.e., that resulting from the simultaneous interaction of several plant and animal species. Some workers (e.g., Janzen, 1980), however, prefer to restrict the term (and concept) of plant/herbivore coevolution to instances in which a close evolutionary sequence of reciprocal counter-adaptation between one specific plant population and one specific herbivore population ("pairwise coevolution") can be demonstrated:

"Coevolution" may be usefully defined as an evolutionary change in a trait of the individuals in one population in response to a trait of the individuals of a second population, followed by an evolutionary response by the second population to the change in the first. [Janzen, 1980:611]

By "evolutionary change" or "evolutionary response," Janzen refers to genetic change. For example, specific alleles in wheat have been identified which confer resistance to Hessian flies (Mayetiola destructor), and each of which corresponds to a specific fly allele for counter-resistance (Hatchett and Gallun, 1970). While this has been cited as one of the clearer examples of coevolution (Futuyma, 1979; Thompson, 1982), it still does not meet Janzen's (1980) strict criteria, since the frequencies of alleles for resistance in wheat populations have been determined by agricultural geneticists rather than by Hessian fly herbivory. In another well-studied case, Williams and Gilbert (1981) have described the production by certain Passiflora plant species of structures which mimic the eggs of, and inhibit

oviposition by, their specialized herbivores, the Heliconius butterflies. These structures exploit the behavioral tendency of Heliconius females not to oviposit on plants which harbor previously laid eggs, since early-hatching larvae frequently cannibalize subsequently laid eggs. It seems plausible that the evolution of Heliconius feeding specialization on Passiflora, with its increased threat to the plant's fitness, has in turn selected for the counter-evolution of these defensive pseudo-eggs. Note that even here the strict criterion for coevolution will only be met if the butterflies are shown to have a specific counter-adaptation to circumvent the plant's deception, such as the ability to discriminate mimetic eggs from real ones. Although such discrimination has not been directly observed (Gilbert, 1983), it may be inferred from the close degree of resemblance of the mimetic eggs to real Heliconius eggs. Surely the first "mimetic eggs" on Passiflora were crude bits of tissue and their present resemblance to real eggs must have evolved gradually as the butterflies evolved an improved ability to detect the difference (even if they no longer can do so). This example of coevolution emphasizes the importance of integrating direct observations in ecological time with sound inferences about processes that may occur over evolutionary time.

While coevolution has proven to be, both theoretically and empirically, an elusive and controversial concept (e.g., Jermy, 1976), it has fostered a great deal of work on the biochemical interactions of plants and their enemies, especially herbivores. A major

organizing theory attempting to explain the kinds of plant chemical defenses evolved has been provided simultaneously by Feeny (1976) and Rhoades and Cates (1976). This theory, now generally known as "apparency theory," assumes that ephemeral, early successional plant species are more able than long-lived, prominent, late-successional species to escape herbivory spatiotemporally. Such "unapparent" species typically devote a large proportion of their energy and nutrients to rapid growth, therefore presumably allocate less to chemical defense and thus rely on what Feeny (1976) terms "qualitative" chemical defenses. These are compounds which are produced in low concentrations and have specific, highly toxic effects against a broad range of herbivorous species. Examples of such compounds include alkaloids, saponins, cyanogenic glycosides, glucosinolates and non-protein amino acids (reviewed in Rosenthal and Janzen, 1979). In contrast, long-lived, late-successional species are less able to escape in ecological space and time, are "bound to be found" (Feeny, 1976) by herbivores, and are therefore selected for a greater investment in the amounts of chemicals allocated to defense. Because some herbivores will eventually succeed (evolutionarily) in breaching any qualitative defense, these "apparent" plants should be selected to contain chemicals which are sufficiently general in mode of action as to be effective, in a dose-dependent manner, against both polyphagous and oligophagous species. The best-known examples of such a "quantitative" chemical defense are the tannins, a group of polyphenol compounds which, at least in vitro, precipitate proteins

(both dietary and enzymal) and thereby inhibit digestion (e.g., Feeny, 1970; but see Bernays, 1981, and Zucker, 1983, for a growing controversy regarding their in vivo effects).

Several criticisms can be raised against the theory of plant apparency. First, the prediction that early-successional species should invest in qualitative rather than quantitative defenses rests on the assumption that qualitative defenses are less costly to produce than quantitative ones, simply because they are effective at, and therefore produced in, lower concentrations. However, the cost of chemical defense cannot be assessed simply by comparing tissue concentrations when different kinds of compounds are involved. It is not necessarily true that it costs a plant as much to synthesize a milligram of tannin as to produce a milligram of, say, cyanogenic glycoside. Furthermore, some toxins (e.g., alkaloids) may be broken down and resynthesized many times in the same plant (requiring repeated investments of energy), while digestibility-reducers such as tannins may be synthesized only once and retained for life (Fox, 1981). It is not yet known which of these two strategies is the more costly (and this too will surely vary with the chemical nature of the particular compounds involved). There may also be costs associated with the internal transport of the compounds or the production of special sites for their storage (Chew and Rodman, 1979). Thus, while it is reasonable to assume that there is some cost to the plant for chemical defense, the a priori assumption that the cost to ephemeral species is necessarily less than that paid by persistent species is not justified (Fox, 1981).

Second, one should also be wary of the dichotomy between qualitative and quantitative defenses (Feeny, 1976). Toxins may well have dose-dependent effects (e.g., glucosinolates: Chew and Rodman, 1979; sesquiterpenes: Langenheim et al., 1980) and purported digestibility-reducers such as tannins may have little or no effect upon adapted specialists (Fox and Macauley, 1977; Bernays et al., 1980).

Third, apparency itself can be quite an elusive concept. While Rhoades and Cates (1976) base their definition on ecological longevity, growth form, seral stage, etc., Feeny (1976) includes the host-finding ability of the herbivores as well. Rhoades (1979) has argued that if host-finding adaptations are included as a component of plant apparency, the entire concept of apparency is rendered circular. This is because the intensity of selection for host-finding ability should be greater for herbivores attacking rare or ephemeral plants than for those attacking highly apparent plants. Once host-finding ability is included, the apparencies (sensu Feeny) of the two plant types converge. Thus, it is not clear that a short-lived herbaceous plant is any less apparent to its specialized herbivores (which may have sophisticated host-finding adaptations) than is an ecologically more persistent plant species. Yet, in order to make predictions about the kinds of defense to expect among various plant groups, we need to have some relative measure of the selection pressure exerted by the plants' enemies. Clearly, this can only be appreciated by taking into account enemy host-finding abilities.

Given these problems with apparency theory, it may be best to restrict its application to discussions of the defenses of related plants facing similar guilds of enemies. If, for example, most plants within a family utilize similar allelochemicals for defense (Ehrlich and Raven, 1964), then it may be safe to assume that those species producing greater quantities of the allelochemical make greater investments in chemical defense than do related species producing less of the allelochemical. This is surely safer than assuming cost differentials for qualitatively very different compounds in widely divergent plant families (see above). Also, if the enemy guilds are similar for the related plants, one might assume similar host-finding abilities and then concentrate on making predictions about defensive investments based on such considerations as growth habit (e.g., herbs vs. shrubs), population density, and phenology. This approach will be taken in a later section in discussing the chemical defense of milkweed plants.

Specialization and Generalization in Herbivory

It has frequently been suggested that since generalist herbivores use a broader range of resources than do specialists (by definition), they are less susceptible to fluctuations in the availability of any particular food species and are therefore "ecologically resilient" (e.g., Levins, 1968; Schoener, 1970; MacArthur, 1972). They must, however, cope with a broader variety of resource defenses and are therefore thought to be less efficient at utilizing any one particular

resource type than are species which specialize on eating only one or a few resource types (MacArthur, 1972; Feeny, 1975; Scriber and Feeny, 1979). One means by which generalist insects have adapted biochemically to a broad range of dietary toxins is through the mixed-function oxidase (MFO) system (Brattsten et al., 1977). This microsomal system permits the rapid induction, upon contact with specific toxins, of the specific oxidative enzymes required for their detoxification. Krieger et al. (1971) demonstrated that the general level of MFO activity was significantly greater in polyphagous lepidopteran larvae than in oligophagous species.

Such biochemical adaptation is presumably effected at some metabolic cost to the insect, i.e., requiring energy which could instead be utilized for other fitness-promoting activities. Feeny (1975) has conceptualized two kinds of biochemical metabolic costs which may be incurred. The first is the cost of synthesizing appropriate detoxification enzymes and any morphological structures required for their storage. This is the "fixed cost" that is paid regardless of the quantity of toxin encountered. The second kind of cost is the "variable cost" of running the detoxification process itself, the magnitude of which depends upon the quantity (and chemical nature) of toxin to be handled. Feeny (1975) argues that while the variable costs of detoxifying a particular compound are probably similar for both specialists and generalists (since similar processes would be required), the fixed costs should be lower for specialists since they must construct and retain biochemical mechanisms for dealing with a more limited range of toxic compounds. This leads to

the prediction that polyphagous species should utilize any particular food type less efficiently than would oligophagous or monophagous species.

This prediction was confirmed by Auerbach and Strong (1981) who found that specialist insects that feed on Heliconia imbricata leaves had higher food utilization efficiencies than did more polyphagous species also fed H. imbricata. Scriber (1979) also provided data that support the prediction. He showed that larvae of the Lauraceae-specializing butterfly, Papilio troilus, had food utilization efficiencies 2 to 3 times greater than did the more polyphagous P. glaucus when also fed a lauraceous diet. Other studies have failed to confirm the prediction, however. For example, Futuyma and Wasserman (1981) fed leaves of Prunus serotina to the tent caterpillars, Malacosoma americanum (a specialist on Prunus) and M. disstria (a more polyphagous species), finding no significant difference in their food utilization efficiencies. [Fueling the debate further, studies by Schroeder (1976, 1977) and Scriber and Feeny (1979) claim to show that specialists are no more efficient food-converters than are generalists. However, because these authors did not compare efficiencies when the specialists and generalists were fed the same plants, their observations are of limited value in the present context.]

One reason why conflicting evidence may be found in studying differences between specialists and generalists has been suggested by Fox and Morrow (1981). These authors show that many species commonly regarded as "generalists" nevertheless exhibit a considerable degree

of specialization at the population level. Because the evolution of feeding adaptations would normally occur at the population, rather than entire species, level, it is possible that such populations are as biochemically and digestively specialized as are populations of species which feed on a single food type throughout their ranges.

A second possible explanation for the contradictory evidence is suggested by the work of Smiley (1978) who found that larvae of the butterfly Heliconius erato (which is essentially monophagous on Passiflora biflora) grew faster on P. biflora than did larvae of the more oligophagous species H. cydno (which feeds on at least five Passiflora species). However, another monophagous species, H. melpomene, grew no faster on its hostplant (P. oerstedii) than did H. cydno. Smiley (1978) suggests that H. melpomene has specialized on P. oerstedii for ecological rather than biochemical or nutritional reasons. These might include higher interspecific competition or parasitism rates on other available host plant species. If such "ecological monophagy" (Gilbert, 1979) is widespread (see also Singer, 1971), specialists may not necessarily exhibit greater food utilization efficiencies than generalists.

The case of H. erato described above exemplifies a second type of monophagy, in which specific digestive/biochemical adaptations for coping with the primary and secondary chemistry of the particular host species have apparently evolved. Gilbert (1979) terms this "coevolved monophagy," but "evolved monophagy" might be a preferable term since such herbivore adaptation may exist even when "coevolution" per se can not be demonstrated.

While the basic distinction of evolved vs. ecological monophagy is valid, it is probably best to regard these as opposite ends of an evolutionary continuum. For example, if the selection pressures encouraging ecological monophagy persist for sufficient time, then there will presumably also be selection for an increase in the efficiency with which the particular host type is utilized. Thus, what begins as ecological monophagy may well result in evolved monophagy. At any point in evolutionary time, we may expect to find species (or populations) that are at different (and unknown) points along this evolutionary path. This fact will certainly hamper our ability to make predictions about the relative dietary efficiencies of "specialists" and "generalists."

Chemical Defense of Milkweed Plants

The milkweed family, Asclepiadaceae (order Apocynales), provides a good example of an early-successional, herbaceous plant group which is fed upon by several oligophagous insect species. It is comprised of some 1700 species of perennial herbs, vines, and shrubs (Willis, 1931). While the family is predominantly pantropical, 108 species within the genus Asclepias alone have been described from North America (Woodson, 1954). Milkweeds are renowned for their toxic properties, based upon a class of C-23 steroids called cardenolides (Kingsbury, 1964). These typically occur in glycosidal form in the plants (but are not present in all species; Roeske et al., 1976) and are therefore often referred to as "cardiac glycosides." The

designation, "cardiac," refers to the well-known effect of these compounds on vertebrate heart tissue, acting through an inhibition of the Na-K-ATPase system (Hoffman and Bigger, 1980). In therapeutic dosages, cardenolides cause the heart to beat more slowly and regularly, but in excessive dosages, they are lethal (Hoffman and Bigger, 1980).

Although the toxic effects of cardenolides to many species of domestic mammals are well-established (e.g., Detweiler, 1967), their toxicity to invertebrate herbivores is less clear. This is a serious gap in our knowledge because there exist several groups of herbivorous insects that specialize on asclepiads (Duffey and Scudder, 1972; Scudder and Duffey, 1972; Rothschild and Reichstein, 1976) and it has frequently been inferred that these have "coevolved" with milkweed plants after breaching the presumed cardenolide defense (see, e.g., Brower and Brower, 1964; Dixon et al., 1978; Harborne, 1983). Thus, it is obviously crucial to know whether cardenolides are, in fact, toxic to herbivorous insects. The evidence for this will be reviewed in detail in a later section.

While qualitative variation in cardenolide contents, within and among milkweed populations, has been noted (e.g., Brower et al., 1982), little effort has been made to relate such variation to ecological and evolutionary forces. Roeske et al. (1976) summarize work on the individual cardenolides isolated and identified from various milkweeds and show that some populations of Asclepias curassavica and A. syriaca contain specific cardenolides not reported from other populations (see also Malcolm, 1981). Also, in A.

eriocarpa, the relative proportions of the various leaf cardenolides appear to vary between populations. The authors do not speculate on the ecological significance of such differences. However, because the toxicity of most allelochemicals is positively related to their lipophilicity (e.g., Harborne, 1983), this may suggest that those populations containing more lipophilic cardenolides face more resistant (more "highly coevolved"?) herbivores than do populations having more hydrophilic (i.e., polar) compounds. This argument could be extended to cover interspecific qualitative differences as well (see below).

Quantitative variation in cardenolide concentration within populations has also been described (e.g., Nelson et al., 1981; Brower et al., 1982). One of the most variable species is Asclepias syriaca, which may exhibit a 40-fold range of cardenolide concentration within a single population (Roeske et al., 1976). Other populations of this species are reportedly devoid of cardenolides altogether (e.g., Rothschild et al., 1975). The ecological significance of such differences has never been studied and it would be instructive to learn, for example, whether individuals or populations lacking or low in cardenolide face a different set of herbivores than do those having higher tissue concentrations. [It is possible that some plants lacking cardenolides compensate for this by the storage of other allelochemicals. Although both saponins and alkaloids reportedly occur in at least some milkweeds (see Brower and Brower, 1964), their functions in these plants remain totally unstudied.]

Although nearly all milkweed species examined have leaf cardenolide concentrations less than 1% of dry weight (Roeske et al., 1976), interspecific variation should not be ignored. Several species apparently lack cardenolides entirely, or contain undetectable amounts (e.g., Gonolobus spp., Brower, 1969; A. viridiflora, Roeske et al., 1976; Hoya, Stephanotis, Marsdenia, Tylophora, Cynanchum, Gymnema, Rothschild and Marsh, 1978). The most complete tabulation of milkweed cardenolide concentrations is provided by Roeske et al. (1976: Table 2) for 24 species, and well illustrates the wide interspecific variation. The highest recorded cardenolide concentration in leaves is 14.7% of dry weight for A. masonii from California (Roeske et al., 1976; but this is based upon a single determination and should be replicated).

How can these differences be explained? Using apparency theory, one might predict that the most apparent species would, other factors being equal, tend to have the highest cardenolide concentrations, whereas the least apparent would have the lowest. Roeske et al. (1976), using Woodson's (1954) data on species distributions and density, have found that those species having wide geographic distributions and growing densely tend to have low cardenolide concentrations, while those of more restricted distributions, occurring in low density, have higher cardenolide concentrations. These trends are opposite to those expected from apparency considerations, since widespread, densely growing plants are presumably more apparent than restricted, sparsely growing plants.

However, the abundance and density of milkweed plants have probably changed drastically with the development of North American agriculture (e.g., Fink and Brower, 1981), and so Woodson's (1954) biogeographic (county record) information may no longer be valid for making quantitative comparisons.

It is noteworthy that, of those milkweed species growing in north-central Florida, only two of six studied produce leaf crops by early spring (Cohen, unpubl. data), when large numbers of asclepiad-specializing monarch butterflies re-enter eastern North America from overwintering sites in Mexico (Urquhart and Urquhart, 1977). These two species, Asclepias humistrata and A. viridis, tend to grow in dense patches, and were located in each of the years 1981-1983 by the migrants and utilized heavily as larval food sources (Cohen, pers. observ.). Both species have relatively high leaf cardenolide concentrations (Table 1). In contrast, the remaining four species (A. tuberosa, A. amplexicaulis, A. tomentosa, and A. verticillata) grow more sparsely, produce leaf crops too late for use by most migrant monarchs, and have very low leaf cardenolide concentrations (Table 1). While monarchs are surely not the only herbivores attacking milkweeds in north-central Florida (see, e.g., Chapter IV), their massive migrations into the area may provide a more acute risk than the more regular, but lower level, herbivory by other species.

If the quantitative cardenolide differences within or among milkweed species are indeed the result of differential risks to herbivorous insects, it would suggest either that (1) some insects are affected by cardenolides in a dose-dependent manner and, through

Table 1: A survey of midstem leaf cardenolide concentrations for six milkweed species in north-central Florida. Data are expressed as digitoxin equivalents (see Brower et al., 1972) as a percentage of dry weight of leaf tissue.

Species	$\bar{X}$ (%)	Range	N
<u>Asclepias humistrata</u> ¹	0.55	0.36-1.01	12
<u>A. viridis</u> ²	0.38	0.19-0.54	10
<u>A. tuberosa</u> ³	0.02	0.00-0.05	4
<u>A. amplexicaulis</u> ⁴	0.03	0.01-0.04	2
<u>A. tomentosa</u> ⁵	0.06	0.03-0.09	3
<u>A. verticillata</u> ⁶	0.05	---	2

¹ Route 24, ca. 8 km W of Gainesville (Alachua Co.) city limit; collected 4 May 1981.

² ca. 1 km from Alt. Route 27, ca. 3 km W of junction Route 41, Williston (Levy Co.); 18 April 1981.

³ Route 24, at E. city limit Bronson (Levy Co.); 2 July 1981.

⁴ Devil's Millhopper State Geological Site, NW 53 Ave, ca. 1 km W. Gainesville city limit; 2 June 1981.

⁵ NW 62 St., ca. 1 km W of Gainesville city limit; 7 June 1981.

⁶ Route 24, at E. city limit Bronson; 26 May 1981; leaves pooled from two plants for a single cardenolide determination.

coevolution, have selected for higher defensive levels in their hostplants, and/or (2) different insect species have different threshold tolerances for cardenolides and those plant species having greater concentrations may be adapted for defense against more cardenolide-resistant herbivore species.

The Metabolic Cost of Cardenolide Ingestion

The two propositions above require that at least some herbivorous insects be adversely affected by ingested cardenolides. Indeed, only if it can be convincingly demonstrated that these compounds do present a chemical barrier to herbivory by at least some species will it become credible to think of milkweed-specialization by such oligophagous groups as danaid butterflies (Brower, 1969; Brower et al., 1975; Rothschild and Marsh, 1978) and lygaeid bugs (Scudder and Duffey, 1972) as the possible result of a coevolutionary process involving the breaching of such a barrier. Moreover, it should be possible to demonstrate that milkweed-specialists have cardenolide-tolerance capacities exceeding those of either polyphagous species or species that specialize on cardenolide-free plant families.

Considerable attention has been paid to the question of whether milkweed specialists suffer any negative physiological consequences (which are here collectively called "metabolic costs") of ingesting, metabolizing, detoxifying, or sequestering cardenolides. In contrast,

very little effort has been expended in testing the effects of these compounds upon polyphagous species, despite the expectation that generalists should be more likely than specialists to be affected by toxins (see above; also Blau et al., 1978). Nevertheless, the first suggestion of such a metabolic cost in an adapted specialist was made by Brower et al. (1972), who found a progressive southward decrease in the mean cardenolide concentration of migrating monarch butterflies and suggested that those individuals having high cardenolide contents may have had reduced viability or migratory ability relative to those of lower cardenolide content. Rothschild et al. (1975) offered an alternative hypothesis, suggesting that larvae developing in colder, more northern locales would simply require a longer development time than those in warmer, southern areas. As a result, they might ingest more food and, consequently, more cardenolide. This suggestion was later refuted by Dixon et al. (1978) who reared monarch larvae in the laboratory at either 17° C or 28° C. Although those reared at 17° took longer to complete development, they consumed less food, and incorporated into their tissues both the nutrients and the dietary cardenolide more efficiently, than did larvae reared at 28°. As a consequence, rearing temperature had no significant effect upon final cardenolide content of the insects.

A third possibility, not previously considered, is that the lower cardenolide content of southern monarchs is due to a greater degree of wing-scale loss during migration. It has been shown that the cardenolide concentration in the wings is approximately twice that in

the remainder of the body (Brower and Glazier, 1975), and nearly 30 per cent of this wing cardenolide is localized in the wing-scales (Nishio, 1980). It therefore seems possible that the further south a monarch flies, the more scales it loses, and the lower its cardenolide content becomes.

A second suggestion of metabolic costs of cardenolide ingestion was provided by Brower and Moffitt (1974), who found a significant negative correlation between the amounts of cardenolide stored by and the sizes and dry weights of monarch females collected in Massachusetts. The correlation approached significance for males (which, on average, were 9 per cent lower than females in cardenolide concentration) but disappeared entirely among both males and females collected in California. On average, the California butterflies were 62 per cent lower in cardenolide concentration than were the Massachusetts butterflies. It is thus possible that the purported metabolic cost was seen only among the Massachusetts females because only they contained sufficient concentrations to be affected. Massachusetts males came next closest to exhibiting the effect, perhaps because they contained the second highest cardenolide concentrations, and California butterflies exhibited no apparent cost because both sexes contained insufficient concentrations. Future analyses of this problem should attempt to separate true geographic and gender differences from cardenolide concentration differences with which they may be correlated. It would also be valuable to learn whether the apparent cardenolide/body size trade-off phenomenon extends to other milkweed-feeding species, or is limited to monarchs.

Several recent studies have shown that monarchs (Erickson, 1973), African queen butterflies (Danaus chrysippus; Smith, 1978) and milkweed bugs (Oncopeltus fasciatus; Isman, 1977; Chaplin and Chaplin, 1981) tend to grow better when fed those plant species high in cardenolide than when fed those low or lacking in cardenolide. These authors (see also Blum, 1981) interpret such results as evidence against a metabolic cost of cardenolide ingestion. The major fallacy of such an interpretation is the confounding of cardenolide differences among plant species with other nutritionally important species differences. For example, other factors being equal, one might expect those plant species high in nitrogen or water (two important nutrients for insects; see Scriber and Slansky, 1981) to be more desirable foodplants than those low in these nutrients. They should therefore be under stronger selection for chemical (and other) defense. Thus, we might expect a correlation between allelochemic content and nutritional quality (e.g., furanocoumarins: Berenbaum, 1981), and the fact that some insects may grow better on high cardenolide plants may be due to the correlated higher nutritional quality of such plants, and may occur despite any metabolic costs that may still be paid.

For this reason, a solution to the problem of metabolic costs cannot be gained from studies in which several species of hostplant, each having different cardenolide (and nutrient) contents, are fed to insects. Rather, one must add purified cardenolide(s), preferably in varying dosages, to diets of standardized nutritional quality (e.g.,

artificial diets). This has recently been done for monarchs fed cardenolide-free milkweed leaves (Gonolobus rostratus) to which were added increasing concentrations of the cardenolides calotropin, uscharidin, uzarigenin, or digitoxigenin (Seiber et al., 1980). No effects upon larval development time, food consumption, or body weight were identified at any dose level for any of the four cardenolides. The only suggestion of a metabolic cost was a dose-dependent melanism occurring in larvae fed uzarigenin. In Lepidoptera, abnormal melanism is a common indicator of stress such as crowding or starvation (Peters and Barbosa, 1977). However, Seiber et al. (1980) housed their larvae individually and showed that uzarigenin-fed larvae consumed as much food as controls, so that crowding and starvation cannot explain the result. The melanistic response occurred only at dosages 4 to 61 times greater than that found in the hostplant (A. curassavica) from which the uzarigenin was isolated. Thus, while there may be a dose at which a cost is paid, it is rarely (if ever) encountered in nature and it is unlikely that the present levels of uzarigenin in A. curassavica are attributable to herbivore pressure from monarchs.

Seiber et al. (1980) also found a quantitative regulation by monarch larvae of the amount of cardenolide stored in their tissues. Thus, when fed low-cardenolide diets, proportionally more cardenolide is sequestered than when high-cardenolide diets are fed (see also Brower et al., 1982). As a result, the variation in the total amount of cardenolide sequestered is damped. Although the mechanism underlying this regulation has not been investigated, one may wonder why the insects do not simply store as much cardenolide as they

ingest, or do not excrete (or metabolize to noncardenolide compounds) a constant fraction of the ingested cardenolide. Again, there is a suggestion that there may be an upper limit on monarch cardenolide tolerance.

Using in vitro preparations, Vaughan and Jungreis (1977) found that monarch neuronal tissues were some 300 times less sensitive to cardenolide- (ouabain-) induced inhibition of the Na-K-ATPase system than were tissues of two polyphagous lepidopteran species (Hyalophora cecropia and Manduca sexta). This study is important because it represents the first serious attempt to ascertain whether a metabolic cost may be paid by species not adapted as milkweed specialists. It must be regarded with caution, however, because in vitro preparations circumvent a good part of the potentially protective physiology of the organisms. For example, it is possible that the digestive systems of Hyalophora and Manduca prohibit assimilation of ingested cardenolides into the hemolymph. If so, one would not expect the evolution of protection at the neuronal level. Since injection bioassays are equally unrealistic, this caveat also applies to the results of von Euw et al. (1967) and Rafaeli-Bernstein and Mordue (1978) that milkweed-feeding orthopteran species are less sensitive to injections of cardenolide than are species that do not feed on milkweeds. What remains to be done is a comparative study of the effects of ingested cardenolide upon the growth and metabolism of both milkweed-adapted and non-adapted herbivorous insects.

In this dissertation, I present the results of several studies bearing on the question of the metabolic costs of cardenolide ingestion in lepidopteran species that are either adapted or not adapted to feeding on milkweed plants. The specialized milkweed-feeders studied were the monarch butterfly (Danaus plexippus), the queen (Danaus gilippus), and the dogbane tiger moth (Cycnia tenera; Arctiidae). The fall armyworm (Spodoptera frugiperda; Noctuidae) was studied as an example of a highly polyphagous herbivore. Finally, the velvetbean caterpillar (Anticarsia gemmatilis; Noctuidae) was studied as an example of a specialist species which feeds on plants lacking cardenolide (in this case, only plants in the family Leguminosae are eaten). The species selected represent a wide taxonomic range (i.e., both moths and butterflies).

In Chapter II, I discuss the hostplant selectivity and larval success of monarch butterflies in relation to plant cardenolide concentration. If there is, in this species, a metabolic cost of feeding on high cardenolide diets, then ovipositing females might be expected to have evolved a preference for lower cardenolide plants over higher ones. Furthermore, if the costs accrue in the larval stage, then the survival of larvae on high-cardenolide plants might be reduced relative to that of larvae on lower-cardenolide plants. Alternatively, if no significant cost exists, females might instead behave in such a way as to maximize the opportunities of their offspring to sequester defensive chemicals, i.e., higher cardenolide plants should be preferred. Finally, if larvae on high-cardenolide plants are better protected from parasites and predators than those on

low-cardenolide plants, a differential in larval survivorship should be measurable.

Chapter III presents a comparative analysis of the cardenolide contents of wild queen butterflies (D. gilippus berenice) collected from three locations in Florida, and of monarchs from one of those sites. Since it is known for other lepidopteran species that body size is positively correlated with fecundity (Hinton, 1981), the hypothesis that these danaid species pay fitness costs of cardenolide ingestion would be supported if negative correlations between butterfly cardenolide concentration and body size or weight were found. In a separate experiment, also reported in Chapter III, these two species were simultaneously reared in the laboratory on the milkweed, Asclepias humistrata, and their cardenolide sequestration abilities compared. Again, correlations between body size, weight, and cardenolide content were sought.

It should be noted that even if such indications of metabolic cost are found, this need not imply that the net benefit to the insect is necessarily negative. Sequestered allelochemicals may provide a chemical defense that outweighs any costs paid to acquire them. As a result, there may be a net gain in fitness. Nevertheless, a reduced body size may be considered an investment made in order to achieve this level of defense. It is assumed that, as adaptation to allelochemicals evolves, the magnitude of the investment required would be reduced.

In addition to their relevance to the issue of metabolic costs, the data on wild queens provide the first practical application of the

chromatographic cardenolide "fingerprinting" technique devised by Brower et al. (1982) to identify the hostplants of wild-caught danaid butterflies. The results may also shed some light on a long-standing problem in the study of mimicry, viz., why the viceroy butterfly (Limenitis archippus) in Florida mimics the queen instead of its usual model, the monarch (e.g., Brower, 1958b).

In Chapter IV, I investigate the issue of cardenolide sequestration in an oligophagous arctiid moth, Cycnia tenera. This is an aposematic, apocynad/asclepiad-specializing species reported by Rothschild et al. (1970) not to sequester cardenolides from its hostplant (Asclepias syriaca in their study). This raises the interesting question of whether, even among specialists, some species do not sequester cardenolides because they are more sensitive to these compounds than are sequestering species such as danaids. Unfortunately, Rothschild et al. (1970) did not confirm that the A. syriaca fed to the C. tenera larvae indeed contained cardenolides. It is thus necessary first to either confirm or refute their findings, using hostplants of known cardenolide contents. In this chapter, I report on the growth, fat storage, and both qualitative and quantitative aspects of the cardenolide contents of C. tenera reared on the milkweeds, A. humistrata and A. tuberosa.

In Chapter V, I report the results of a series of gravimetric experiments (Waldbauer, 1968) designed to test whether dietary cardenolides per se affect consumption rates, growth rates, body size, and other important fitness indices of larval Lepidoptera. I will compare the metabolic responses to ingested cardenolide of a milkweed

specialist (monarchs), a polyphagous species (fall armyworms), and a species specializing on cardenolide-negative plants (velvetbean caterpillars).

Chapter VI summarizes the major results presented in the dissertation and discusses their relevance to the theories of plant/herbivore coevolution and chemical defense.

CHAPTER II

OVIPOSITION AND LARVAL SUCCESS OF WILD MONARCH BUTTERFLIES (LEPIDOPTERA: DANAIIDAE) IN RELATION TO HOSTPLANT SIZE AND CARDENOLIDE CONCENTRATION*

Introduction

Larvae of the monarch butterfly (Danaus plexippus L.) feed upon milkweed plants (Asclepiadaceae), from which they may ingest and sequester plant defense chemicals known as cardiac glycosides or cardenolides (Parsons, 1965; Reichstein et al., 1968; Brower, 1969; Roeske et al., 1976). In turn, these chemicals are important in defending the monarch against some vertebrate predators by making the butterflies unpalatable (Brower and Brower, 1964; Brower, 1969). Therefore, one might expect the presence, concentration, or particular types of cardenolides present in individual milkweed plants to be among the factors influencing a monarch female's decision to oviposit

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on a particular plant. Dixon et al. (1978) investigated this question on an interspecific level by comparing the numbers of eggs laid on greenhouse plants of three milkweed species differing in cardenolide concentration. They reported an oviposition preference for the species having the lowest cardenolide concentration (Asclepias curassavica), although they concluded that factors such as plant age and the presence of previously laid eggs and larvae are more important determinants of oviposition. However, their study did not test the independent effect of cardenolide concentration upon oviposition preference for the following reasons: 1) milkweed species differ in many ways other than cardenolide concentration, such as water and nitrogen content (Erickson, 1973) or plant morphology (Woodson, 1954) so that ovipositional preferences for a particular species cannot be attributed to any one of these plant differences unless more controlled studies are done; 2) the data on cardenolide concentrations were not derived from the same individual plants upon which the butterflies oviposited, and significant intraspecific differences in milkweed cardenolide concentrations occur (Brower et al., 1982; and this study); and 3) the study was carried out in captivity where unnatural butterfly behavior commonly occurs (e.g., Dixon et al., 1978:443, 448).

To avoid the above problems, I carried out field observations of oviposition and larval success of wild monarchs on individual Asclepias humistrata Walt. plants which were then analyzed for variation in cardenolide concentration.

Methods

Ten plants of Asclepias humistrata (Asclepiadaceae), growing along a sparse grassy roadside (Route 346) near Cross Creek, Alachua Co., Florida, were marked for study. The total leaf area of each plant was estimated as follows. A separate collection of 25 A. humistrata leaves was first used in determining the relationship between leaf length and leaf area. Lengths were measured to the nearest 0.1 cm with a ruler and areas determined electronically (to the nearest cm^2) with a Li-Cor Model 3100 Area Meter. The regression relationship was: $\text{area} = 6.34 [\text{length}] - 15.14$, with an $r^2 = 0.92$. Because A. humistrata has opposite leaves of approximately equal size, I then measured (in situ) the length of the least eaten of each pair of leaves on the 10 marked plants. Their leaf areas were then estimated using the regression formula, summed for each plant, and then doubled to provide an estimate of the total plant leaf area.

I counted the total numbers of monarch eggs and larvae on each plant on each of the following dates in April 1981: 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, and 23. These total counts overestimate the actual egg and larval numbers since each individual may be counted more than once (i.e., on different dates). However, this problem applies equally to all 10 plants and thus does not affect the statistical analyses or conclusions. I therefore define larval success as the total number of fifth instars per egg counted.

On the day final counts were made, I collected for cardenolide analysis four midstem leaves from each of the nine surviving plants, avoiding any partially eaten leaves. To assess whether this collection of only uneaten leaves would bias the results (i.e., if their cardenolide concentrations were not representative of the plants' foliage in toto), two partially eaten and two uneaten midstem leaves were also taken from each of seven adjacent plants. All leaves were then sealed in plastic bags, placed on ice, and kept in a deep-freeze until analysis in mid-July. Cardenolide concentrations (expressed as micrograms of digitoxin equivalent per 0.1 g dry weight of leaf) were determined by standard spectrophotometric methods (see Brower et al., 1972, 1975).

Tests of association between variables were done with Spearman's rank order correlation, followed by t-tests for significance.

Results

The numbers of eggs and larvae, larval success rates, plant sizes, and cardenolide concentrations for each plant are shown in Table 2. Egg densities were also calculated in order to assess the influence of cardenolide concentration, independent of plant size, upon oviposition. It is evident from these data that a large percentage of eggs ($\bar{x}=96\%$) failed to reach the fifth instar. Figure 1 shows that a constant (43%) proportion of the larvae appear to be lost, presumably to mortality, during each instar.

Table 2. Eggs, larvae, larval success, and cardenolide concentrations for 10 *Asclepias humistrata* plants.

Plant	No. stems	No. leaves	Est. total leaf area (cm ²)	Total no. eggs	Egg density (eggs/100 cm ²)	Total number larvae					Percent larval success (No. larvae/egg) $\times 100$					Cardenolide concentration (μ g 0.1 g dry weight leaf)
						I	II	III	IV	V	I	II	III	IV	V	
1	1	12	362	8	2.21	4	2	1	0	0	50	25	13	0	0	438
2	2	18	322	21	6.52	5	8	3	3	2	24	38	14	14	10	205
3	1	11	300	7	2.33	3	0	0	0	0	43	0	0	0	0	560
4	1	8	202	27	13.37	4	0	0	0	0	15	0	0	0	0	602
5	1	8	122	5	4.10	3	0	0	0	0	60	0	0	0	0	415
6	11	77	1976	92	4.66	53	35	17	10	7	58	38	18	11	8	279
7	3	32	699	83	11.74	21	10	5	2	3	25	12	6	2	4	374
8	2	26	655	35	5.34	15	7	1	0	0	43	20	3	0	0	496
9	3	42	1250	24	1.92	4	10	5	5	2	17	42	21	21	8	601
10	7	64	1510	25	1.66	21	20	17	11	3	84	80	68	44	12	*
$\bar{x}$	3	30	740	33	5.39	13	9	5	3	2	42	26	14	9	4	441
SD	3	24	630	31	4.12	16	11	7	4	2	49	44	35	29	20	140

* Not determined because plant died before termination of study.

Source: Cohen and Brower, 1982

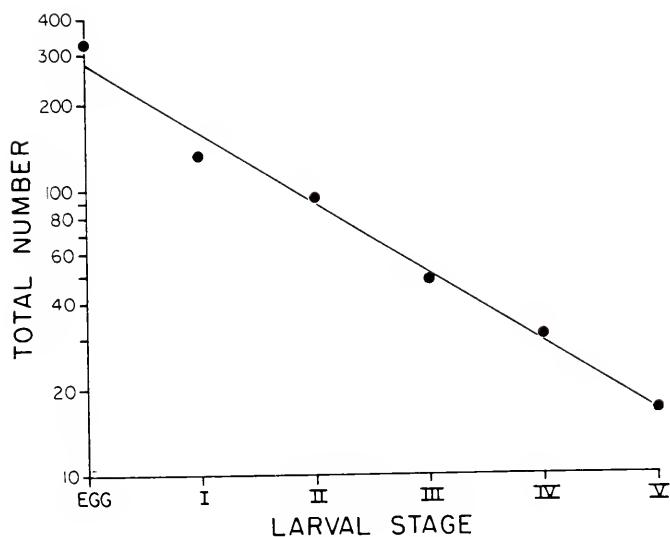


Fig. 1. The number of monarch eggs and larvae of each instar on 10 *Asclepias humistrata* plants, pooled over 11 observation days. The least-squares regression line suggests a constant 43% mortality rate at each instar. (Source: Cohen and Brower, 1982)

Cardenolide concentrations within the plant population varied approximately three-fold (coefficient of variation = 32%; Table 2). Since no significant difference in the cardenolide concentrations of uneaten and partially eaten leaves was found (Table 3), the analysis of only uneaten leaves from the marked plants was not biased by this factor.

Significantly more eggs were laid on larger plants, with the consequence that egg densities did not vary with plant area (Table 4). Fifth instar larval success was positively correlated with plant size. However, the number of eggs laid per plant, egg densities, and larval success rates all varied independently of the cardenolide concentrations of the plants.

Discussion

I found a positive correlation between the number of eggs on a plant and its total leaf area. This is expected on purely statistical grounds since larger plants present larger targets for oviposition. A consequence of this relationship is that egg density does not vary regularly with plant size (Tables 2 and 4). It may be surprising, therefore, that larval success does correlate with plant size, since this is not likely to be due to overcrowding and competition among larvae for food or space. I suggest that larger plants, having more overlapping stems and leaves, may offer greater protection against

Table 3. Cardenolide concentrations (ug/0.1g dry weight) for 7 matched sets of partially eaten (PE) and uneaten (UE) Asclepias humistrata leaves.

	P L A N T							$\bar{x}$	SD
	A	B	C	D	E	F	G		
PE	390	497	681	205	723	434	513	492	176
UE	395	639	613	231	211	807	438	476	221

$t=0.15$; $P>0.05$; Student's t -test, matched pairs design. Variances are not significantly different: $F_{\max}=1.26$; $P>0.05$. (Source: Cohen and Brower, 1982)

Table 4. Correlation coefficients for monarchs and plant area, and for monarchs and cardenolide concentration. Egg numbers and larval success vary positively with plant area, but no correlations with cardenolide concentration occur.

	PLANT AREA (N=10)	P	CARDENOLIDE CONCENTRATION (N=9*)	P
TOTAL NO. EGGS	0.66	0.04	-0.17	NS
EGG DENSITY	-0.35	NS	-0.20	NS
V LARVAL SUCCESS	0.69	0.03	-0.25	NS

Spearman rank order correlations; $\alpha=0.05$.

* No cardenolide data available for plant 10 which died.

(Source: Cohen and Brower, 1982)

both biotic (e.g., parasites and predators) and physical (e.g., direct sunlight and dessication) mortality factors. The curve in Fig. 1 suggests a constant proportional mortality rate. However, this is based upon a mixed cohort of eggs and larvae of varying initial ages, as well as some recruitment, summed over the 19 day period. Also, unlike monarchs in California (Brower et al., 1982), these fifth instars usually left their hostplants when ready to pupate, and thus a few may have been missed. The data may therefore slightly underestimate fifth instar larval success. Yet, despite these error factors, it is remarkable that the data fit so well the logarithmic relationship characteristic of Type II survivorship curves (Wilson and Bossert, 1971).

No relationship between egg numbers or egg densities and the cardenolide concentrations of the plants was found. This is in contrast to the apparent oviposition preference for low-cardenolide plant species reported by Dixon et al. (1978). Moreover, the relatively high intraspecific variance in plant cardenolide concentrations (Table 2), together with similar variability found by Brower et al. (1982) for A. eriocarpa, reaffirms the necessity of measuring concentrations in the particular plants for which oviposition data are gathered, rather than generalizing for entire species or even populations. Intraplant variation is also possible (e.g., Rhoades and Cates, 1976). I have investigated the specific hypothesis that intraindividual cardenolide differences exist between uneaten and partially eaten leaves, but no such differences were found (Table 3).

I measured plant sizes on 4 April but collected leaves for analysis on 23 April. Plant sizes change over time as a function of both herbivory and growth. Similarly, cardenolide concentrations may vary with time (e.g., Nelson et al., 1981, for A. eriocarpa). However, my conclusions are based on rank-order, rather than parametric, correlations and thus require only the assumption that size and cardenolide rankings of the plants did not change over the course of the 19-day study. At least for plant size, this accords with subjective observations of the plants.

Brower et al. (1982) demonstrated that monarchs reared on low-cardenolide A. eriocarpa plants sequester proportionately more cardenolide than do those reared on high-cardenolide plants. Thus, the monarchs effectively regulate their own chemical concentrations, with the resultant variation in butterfly concentrations considerably less than that in the plants upon which they had fed. This regulatory ability suggests that the actual cardenolide concentration of the plant on which an egg is laid is, within certain limits, of little significance in determining the ultimate amount of cardenolide in, and hence probable degree of protection of, the butterflies. However, this regulatory ability appears to break down when plant species of extremely low cardenolide content are eaten (Brower, Seiber, and Nelson, in prep.). Since A. humistrata is a relatively high-cardenolide species (see Roeske et al., 1976), the possibility remains that females would be more selective in choice of hostplant when ovipositing on lower-cardenolide species.

The lack of correlation between plant cardenolide concentration and monarch egg deposition patterns or larval success rates provides evidence against the hypothesis that specialist herbivores pay metabolic costs of feeding on cardenolide-containing plants. These are, however, but two possible measures of such costs, utilizing only one specialist insect species. In the next chapter, I will examine (inter alia) the relationship between the amount of cardenolide sequestered by two specialist species and their body sizes and weights. Since the latter variables are correlated with fecundity in most Lepidoptera (Hinton, 1981), this will constitute a further (indirect) test of the metabolic cost hypothesis. Moreover, since it is unlikely that all herbivores are equally well adapted to plant allelochemicals (perhaps reflecting different degrees of coevolution?), the addition of a second species will help provide a comparative basis for understanding the evolution of such adaptations.

CHAPTER III

DIFFERENCES AND SIMILARITIES IN THE CARDENOLIDE CONTENTS OF QUEEN AND MONARCH BUTTERFLIES IN FLORIDA AND THEIR ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

Introduction

The butterfly family Danaidae has figured prominently in the development of the theory of plant/herbivore coevolution (Brower and Brower, 1964; Ehrlich and Raven, 1964) and has provided one of the best examples of the chemical defenses of insects against vertebrate predators (Brower, 1969; Brower et al., 1982; Dixon et al., 1978; Reichstein et al., 1968). However, much of our understanding of the ecology and evolution of plant/danaid/predator interactions is based upon only one species in this tropical family, the monarch butterfly (Danaus plexippus L.; review in Brower, in press). Because this species is known to be unique among danaids in certain other respects (e.g., migration: Urquhart, 1960; lack of plant-derived sex pheromones: Edgar et al., 1976; Boppré, 1978), one must question whether monarchs are representative of the Danaidae with respect to hostplant adaptation and chemical defense, or whether they represent just one point (or range) within a broader spectrum of danaid adaptations. If such a spectrum is found to exist, it may ultimately

become possible to reconstruct some of the evolutionary steps leading to the close association between danaids and their highly toxic milkweed hostplants (Asclepiadaceae).

Danaid species differ in the amounts of toxic cardenolides which they sequester from their foodplants. For example, Brower et al. (1975) showed that the cardenolide concentrations of laboratory-reared African queen butterflies (Danaus chrysippus L.) were only about 30 per cent that of monarchs simultaneously reared on the same milkweed hostplants (see also Brower et al., 1978; Rothschild et al., 1975). The cardenolide concentrations of queen butterflies (D. gilippus berenice Cramer) from Florida were, on average, about 75 per cent that of monarchs reared in the laboratory on the same hostplants. Other genera of danaids (e.g., Amauris, Euploea) appear to prefer as larval hostplants those milkweed species lacking cardenolides (Rothschild and Marsh, 1978).

Does this diversity in the storage of cardenolides reflect differences among danaid species in their degree of adaptation to these defensive plant allelochemicals? Do queens, for example, store less cardenolide than monarchs because they are "less coevolved" and can tolerate these compounds less readily? Do the cardenolide contents of various danaid species differ only quantitatively, as described above, or are there also qualitative differences in the particular set of hostplant cardenolides sequestered?

Here, I address these questions through a comparison of the cardenolide contents of wild queen butterflies (D. gilippus berenice)

from three populations in Florida and of monarchs (Danaus plexippus) from one of these populations. Following Brower and Moffitt (1974), I will assess the "cost" of storing cardenolides by searching for correlations between body size or weight and cardenolide concentration of the butterflies. Since body size and weight are typically correlated with fecundity in Lepidoptera (see Hinton, 1981), a negative correlation with cardenolide concentration would suggest that one component of fitness (i.e., fecundity) has been traded for another (e.g., higher survival due to chemical defense from cardenolides). While such a trade may well be of positive net value to the insect, it nevertheless would require an investment or cost which, it is assumed, should be lessened as adaptation to allelochemicals evolves.

The subspecies of queen studied here is of further interest because it is the apparent model for mimicry by the southern subspecies of the viceroy butterfly (Limenitis archippus floridensis Strecker), which throughout the remainder of North America mimics the monarch (Brower, 1958a,b; Klotz, 1951; Remington, 1968). Thus, a comparison of the chemical basis for defense in the queen and monarch should aid in understanding both the selective rationale for the switch in viceroy mimicry and the broader issues of plant/herbivore coevolution mentioned above.

Methods

Wild queen butterflies were collected from three populations in Florida, listed from north to south as follows: Lake Istokpoga, Highlands County, 7 September 1981; Corkscrew Swamp Sanctuary, Collier County, 6 September 1981; Miami, Dade County, 4 December 1981. In addition, monarchs were collected from the Miami site where they were sympatric with queens, and where only the milkweed Asclepias curassavica grew abundantly. (However, a few A. incarnata plants were also located.) These collections therefore permit a study of population variation in cardenolide content of queens, as well as a comparison of the relative value, with respect to cardenolides, of monarchs and queens as models for viceroy mimicry. All butterflies were placed on ice immediately following capture, killed by freezing, and later dried for 16 h at 60° C. Dry weights were determined using a Mettler AK-160 electronic balance. The right wing was removed with forceps and the distance from the apex to the anterior notal process was measured to the nearest 0.5 mm with hand calipers. Fat was removed from the butterflies by petroleum ether extraction of each entire insect for 1 h (methods in Walford and Brower, in prep.). This procedure removes only negligible amounts of cardenolide from the insect (Nelson and Brower, unpublished data; see also Nishio, 1980). Lean weights were calculated by subtracting the weight of extracted fat from the total dry weight of each insect.

Cardenolide content was determined by standard spectrophotometric methods (Brower et al., 1972, 1975), with one modification. Soon

after beginning the spectroassay of queens from Corkscrew Swamp, it became evident that many of the butterflies contained very little cardenolide. In such cases it is frequently difficult to achieve a stable absorbance reading. Consequently 0.3 ml of a 12.5×10^{-5} M ethanolic solution of digitoxin was added to each butterfly extract in the cuvette (replacing 0.3 ml of 95% ethanol; see Brower et al., 1972) in order to artificially "boost" absorbance readings to a more stable midrange. A pilot test demonstrated that the absorbance of the "boost" digitoxin alone was 0.400 ± 0.009 ($\bar{X} \pm$ SD; N=9). This mean value was therefore subtracted from the total absorbance read for a sample, the remainder being the absorbance due to the butterfly extract alone.

In order to compare qualitatively the cardenolides present in sympatric monarchs and queens from the Miami sample, those butterflies containing a total of at least 30 ug equivalents of digitoxin (as determined from the spectroassay) were subjected to a lead-acetate clean-up procedure in preparation for thin-layer chromatography (TLC). The procedure used was that described by Brower et al. (1982) with the exception that the final solution was filtered through a Millipore filter (Millipore Corp., Bedford, MA) rather than through a funnel of glass wool and anhydrous sodium sulfate. This clean-up procedure removes much of the interfering pigments and other noncardenolide compounds from the butterfly samples. TLC was then performed and plates developed four times in a chloroform : methanol : formamide solvent system (90:6:1). Further details of the TLC procedure are available in Brower et al. (1982).

In addition to the wild-caught butterflies, eggs and first instar larvae of both monarchs and queens were collected from milkweed plants growing in La Vega province, Dominican Republic, during July, 1981. These were brought back to the laboratory and reared to maturity on an exclusive diet of A. humistrata leaves, collected wild in the vicinity of Gainesville, Florida. The leaves of this species contain relatively high concentrations of cardenolide (see Chapter I). From these rearings, 10 adult monarchs and 7 queens were compared for quantitative (via spectrophotometry) and qualitative (via TLC) differences in cardenolide storage. For comparison, two arctiid moth species (Cynia tenera, an apocynale specialist; and Estigmene acraea, a highly polyphagous species; Tietz, 1972) were reared on A. humistrata and chromatographed along with the danaiids.

Results

Wild-Caught Butterflies from Florida

The frequency distributions of cardenolide concentration and total cardenolide per insect for the wild-caught butterflies are shown in Figs. 2 and 3, respectively. Each distribution departs significantly from normality (Shapiro-Wilk (W) tests; $p < 0.01$; Helwig and Council, 1979). Thus, nonparametric statistical analyses were used.

Males and females did not differ significantly either in cardenolide concentration or total content in any of the queen or monarch populations studied (Table 5; Wilcoxon 2-sample tests, $p > 0.05$ for all pairwise comparisons). Consequently, the data from both sexes of each population were pooled for all subsequent analyses.

Cardenolide concentration varied significantly among the three queen populations (Kruskal-Wallis $H=31.60$, $df=2$, $p < 0.0001$), as did the total cardenolide content per butterfly ($H=30.03$, $df=2$, $p < 0.0001$). Pairwise comparisons revealed that the queens from Lake Istokpoga had significantly lower cardenolide concentrations than either those from Corkscrew Swamp (Wilcoxon 2-sample test; $Z=5.17$, $p < 0.0001$) or those from Miami ($Z=4.72$, $p < 0.0001$), but that the latter two populations did not differ significantly from one another ($p > 0.05$). Similar results were found for total cardenolide content per butterfly.

Analysis of the Miami samples shows that monarchs had significantly greater cardenolide concentrations (Wilcoxon 2-sample test; $Z=4.82$, $p < 0.0001$) and total contents ($Z=6.12$, $p < 0.0001$) than the sympatric queens. Chromatography reveals a single cardenolide profile common to both species (Fig. 4). This consists of nine spots, including a major one at the approximate R_f of digitoxin, and one of higher R_f . A few individuals (of both species) exhibit all of these spots plus an additional two faint spots of still-higher R_f (numbered spots 10 and 11 in Fig. 4). This chromatogram is virtually identical to that of monarchs reared in the laboratory on Asclepias curassavica (Fig. 4 inset), providing strong evidence that this was the hostplant utilized by the Miami monarchs and queens. To date, no other milkweed

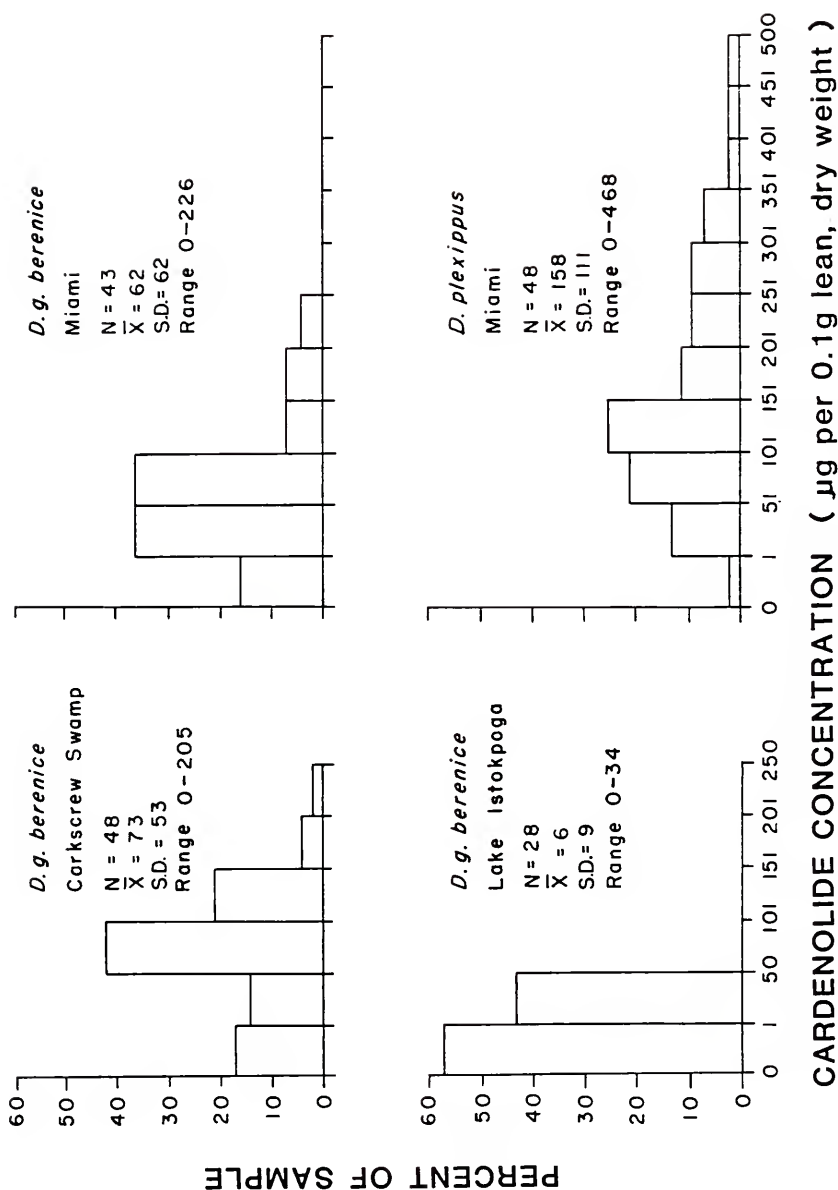


Fig. 2. Frequency distributions of cardenolide concentration (sexes pooled) for three populations of Florida queen butterfly, and one population of monarchs. Units in digitoxin equivalents.

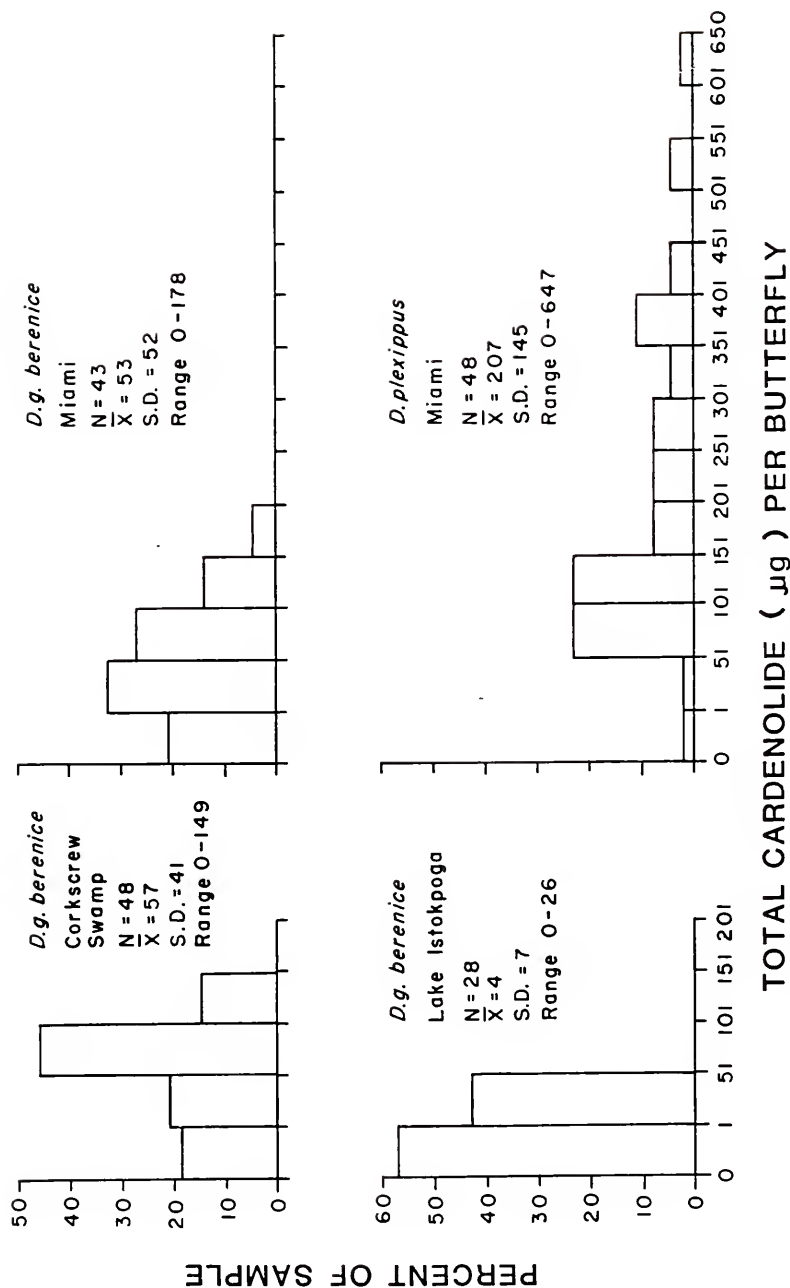
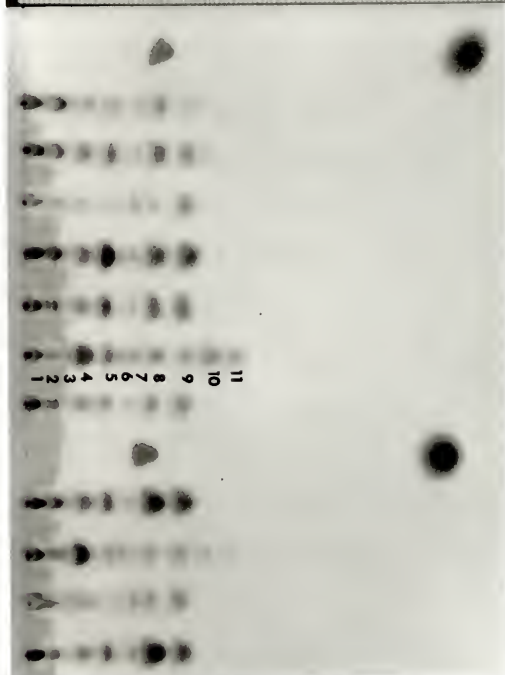


Fig. 3. Frequency distributions of total cardenolide per individual butterfly (sexes pooled).

Table 5. Right wing lengths, weights, fat, and cardenolide contents of wild-caught queen and monarch butterflies from three sites in Florida as follows: Lake Istokpoga (LI), Corkscrew Swamp (CS), Miami (MI).

SPECIES (SITE)	SEX	N	WING LENGTH (cm)		DRY WEIGHT (mg)		LEAN WEIGHT (mg)		PERCENT FAT		CONCENTRATION (ug/.1g)		CARDENOLIDE TOTAL PER INSECT (ug)	
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
QUEENS														
(LI) M		15	4.22	0.19	94.9	22.8	84.4	19.4	10.9	3.0	8	11	6	9
F		14	3.99	0.27	87.8	22.6	78.9	20.4	10.1	3.6	4	5	3	4
(CS)														
M		21	4.27	0.22	95.2	16.4	88.8	15.3	6.7	2.4	64	44	57	42
F		27	4.08	0.25	84.5	15.8	77.1	14.0	8.6	1.8	79	59	58	42
(MI)														
M		26	4.26	0.18	92.4	15.1	82.9	12.1	10.0	3.2	78	71	66	58
F		19	4.24	0.21	107.4	31.6	87.2	16.0	16.4	10.2	37	35	34	31
MONARCHS														
(MI) M		25	4.92	0.29	146.6	28.1	136.6	23.9	6.4	2.6	150	90	198	116
F		23	4.89	0.29	158.8	42.4	133.5	25.4	14.0	9.6	166	131	216	173

Fig. 4. Thin-layer chromatogram of wild-caught monarchs (M) and queens (Q) from Miami area. Developed four times in chloroform:methanol:formamide (90:6:1 v/v/v). The great concordance of all profiles below spot #10 suggests that these animals had all fed upon the same larval hostplant species. Note the agreement of this profile with that of monarchs reared in the laboratory on Asclepias curassavica (Inset), a milkweed growing abundantly in the collection area. Since nearly all milkweed species studied to date produce qualitatively different TLC profiles in danaiids (Brower et al., 1982, in press, and in preparation; see also Fig. 5), A. curassavica is the likely larval hostplant of the Miami butterflies. (Inset from Brower, in press). For reference, a 1:1 (v/v) mixture of digitoxin (dig) and digitoxigenin (dgn) was spotted in three channels. Numbers to the right of each channel indicate the micrograms of cardenolide spotted, calculated prior to lead-acetate clean-up.



Digitoxin/
Digitoxigenin 5
D. plexippus 26

D. plexippus 40

D.g. berenice 51

D. plexippus 75

D. plexippus 40

D.g. berenice 10

D. plexippus 26

Digitoxin/
Digitoxigenin 5
D. plexippus 75

D. plexippus 48

D.g. berenice 40

D. plexippus 75

INSET

D. plexippus

A. curassavica

Digitoxin/
Digitoxigenin

species is known to produce this particular chromatographic profile in danaid butterflies (Brower et al., 1982, in press, and in preparation).

The wing lengths, dry and lean weights, and fat contents of the butterflies are summarized in Table 5. The correlations between these variables and cardenolide concentration are indicated in Table 6. For both male and female monarchs there was a highly significant negative correlation between wing length and cardenolide concentration (Table 6C). Moreover, males also showed negative correlations between cardenolide concentration and both body weight and fat content. For queens, none of the correlations was significant in either sex (all 3 populations pooled; Table 6A).

These statistical differences between monarchs and queens could reflect true species differences. However, the monarchs were all collected in the Miami region, while the queens for this analysis were pooled from 3 areas. It is possible that an unknown geographic effect is operating here, such that butterflies from the Miami region, regardless of species, would show these negative correlations (e.g., due to a common hostplant). To test this, the queens from Miami were also analyzed separately from the other 2 populations (Table 6B). However, as before, no significant correlations emerged ($p > 0.10$ for all tests). Thus, the observed species difference is not attributable to a geographic difference.

It is possible that monarchs show negative correlations between body size and cardenolide concentration, while queens do not, simply

because they store greater concentrations of these chemicals (i.e., queens may not store sufficient cardenolide to be adversely affected by it). If this is the case, then the negative correlations should vanish for that subset of monarchs having concentrations similar to those of queens. To test this, I analyzed only those monarchs (sexes pooled) having cardenolide concentrations equal to, or less than that of, the most highly-concentrated queen (i.e., 226 ug/0.1g). In this case, significant negative correlations between cardenolide concentration and wing length, dry weight and lean weight still occurred (Table 6D). Thus, it appears that the difference between monarchs and queens is not due merely to geographic or cardenolide concentration differences. It is also not due to differences in larval hostplant species, since Fig. 4 demonstrates that both species in Miami had most likely developed on A. curassavica. Rather, the negative correlations appear to represent inherent species differences.

Laboratory-Reared Butterflies (Dominican Republic Stock)

When monarchs were reared in the laboratory on Asclepias humistrata, the females developed significantly higher cardenolide concentrations than did males (Wilcoxon 2-sample test; $Z=2.02$, $p<0.05$; Table 7). No such sex difference was evident for queens reared under identical conditions ($Z=0.53$; $p>0.50$). As in the wild-caught samples, only the monarchs showed significant (again, negative) correlations

Table 6. Spearman correlation coefficients for cardenolide concentration vs. body size, weight, and fat content of wild-caught queen and monarch butterflies from Florida. The data for queens are first shown for all three populations pooled (A), and then for the Miami sample separately (B). Monarchs were first analyzed using the entire data set (C) and then by truncating the set such that only individuals having cardenolide concentrations equal to, or less than, that of the most concentrated queen (226 ug/0.1g) were included (D). See text for explanation.

SAMPLE	SEX	N	CARDENOLIDE CONCENTRATION VERSUS			
			WING LENGTH	DRY WEIGHT	LEAN WEIGHT	PERCENT FAT
QUEENS						
A. (all popu- lations)	Both	119	-0.004	0.01	0.03	-0.14
	M	61	-0.13	0.14	0.13	-0.02
	F	58	0.03	-0.08	-0.08	-0.25
B. (Miami only)	Both	43	-0.17	0.20	0.20	0.14
	M	26	-0.25	0.26	0.25	0.32
	F	17	-0.20	0.32	0.23	0.14
MONARCHS						
C. (Entire Miami Sample)	Both	48	-0.53***	-0.22	-0.21	-0.25
	M	25	-0.55**	-0.44*	-0.39	-0.53**
	F	23	-0.50*	-0.04	-0.10	-0.06
D. (Concen- trations less than 226 ug/0.1g)	Both	35	-0.40*	-0.33*	-0.43**	-0.18
	M	18	-0.36	-0.46*	-0.49*	-0.36
	F	17	-0.38	-0.20	-0.40	-0.06

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.0001$.

between cardenolide concentration and other size and weight parameters (Table 8).

Thin-layer chromatography (Fig. 5) demonstrates that the two danaid species (as well as two arctiid moth species) sequestered virtually identical sets of cardenolides from A. humistrata. This TLC profile is clearly distinguishable from that of butterflies reared on A. curassavica (cf. Fig. 4 inset).

Discussion

Body Size and Cardenolide Content

Brower and Moffitt (1974) reported a negative correlation between the body weight and cardenolide concentration of female monarchs from Massachusetts, and suggested that these individuals may have suffered a "metabolic cost," in terms of growth, of sequestering cardenolides for defense (see also Brower et al., 1972). Such negative correlations were not found for males from Massachusetts (which were 9 per cent lower than females in mean cardenolide concentration), or in either sex collected in California (which were 62 per cent lower in mean concentration than the Massachusetts females). However, these data confound sexual and geographic differences with correlated cardenolide concentration differences (see Chapter I). Here, I have shown that negative correlations between cardenolide concentration and various size and weight parameters, which occur in monarchs, but not

Table 7. Body sizes, weights, fat and cardenolide contents of queens and monarchs (Dominican Republic stock) reared in the laboratory on the milkweed, Asclepias humistrata

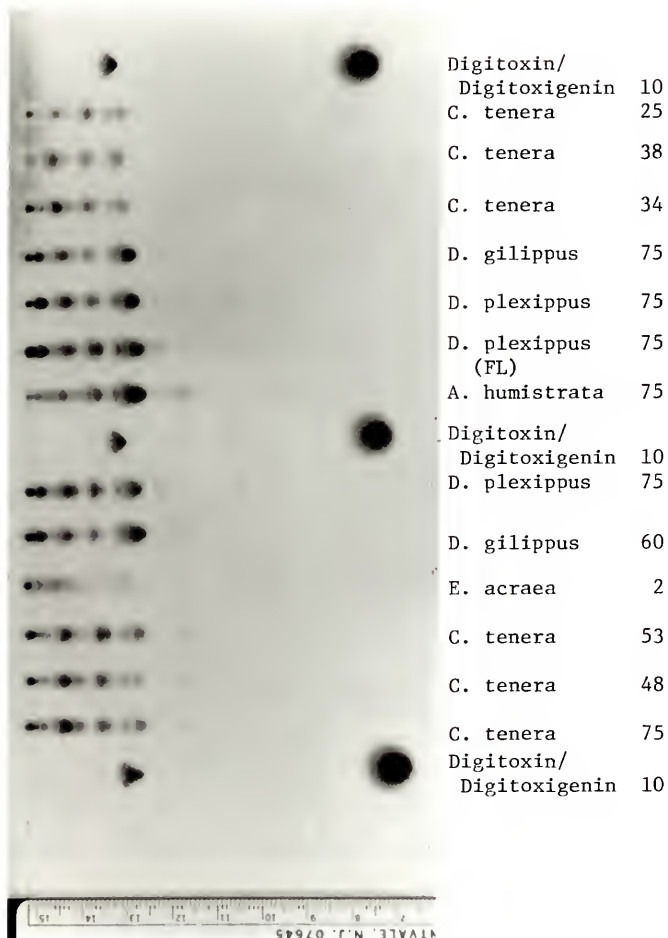
SPECIES	SEX	N	WING LENGTH (cm)		DRY WEIGHT (mg)		LEAN WEIGHT (mg)		PERCENT FAT		CONCENTRATION (ug/.1g)		TOTAL PER INSECT (ug)	
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
QUEEN														
	Both	7	3.60	0.14	77.3	9.0	73.5	9.0	4.9	0.9	368	58	271	61
	M	3	3.63	0.12	82.2	7.7	78.6	8.0	4.5	1.1	341	34	269	55
	F	4	3.58	0.17	73.5	9.0	69.7	8.6	5.2	0.8	388	68	272	74
MONARCH														
	Both	10	4.55	0.18	147.8	31.6	140.3	30.8	5.1	3.1	489	145	657	156
	M	4	4.72	0.05	177.0	28.9	167.8	29.9	5.2	4.9	385	146	615	154
	F	6	4.43	0.12	128.4	12.8	122.1	12.8	5.0	1.7	558	104	684	164

Table 8. Spearman correlation coefficients for cardenolide concentration vs. body size, weight, and fat content of queen and monarch butterflies (Dominican Republic stock; both sexes pooled) reared in the laboratory on Asclepias humistrata.

<u>CARDENOLIDE CONCENTRATION VERSUS</u>					
<u>SAMPLE</u>	<u>N</u>	<u>WING LENGTH</u>	<u>DRY WEIGHT</u>	<u>LEAN WEIGHT</u>	<u>PERCENT FAT</u>
QUEENS	7	0.33	0.18	0.25	-0.57
MONARCHS	10	-0.63**	-0.56*	0.05	-0.58*

* $0.05 < P < 0.10$; ** $P < 0.05$.

Fig. 5. Chromatograms of adult monarchs and queens reared in the laboratory on Asclepias humistrata (developed as in Fig. 4). Note that the profiles of the two species are virtually identical, yet differ from those shown in Fig. 3. All butterflies were collected as eggs or first instars in the Dominican Republic, except for the monarch labelled "FL," which was collected wild in the egg stage in Florida and shows an identical "fingerprint" pattern to those of Dominican Republic stock. For comparison, extracts of 6 dogbane tiger moths (Cycnia tenera) and 1 saltmarsh moth (Estigmene acraea), reared simultaneously on A. humistrata, were spotted on the same silica plate. Also spotted are an ethanolic leaf extract of A. humistrata and a 1:1 (v/v) mixture of digitoxin (dig) and digitoxigenin (dgn). Numbers to the right of each channel indicate the micrograms of cardenolide spotted, calculated prior to lead acetate clean-up.



in queens, are independent of geographic, sexual, foodplant, or correlated concentration differences.

While these negative correlations might well represent "metabolic costs" of cardenolide ingestion, as suggested by Brower and Glazier (1975), there is no direct evidence of a causal connection between cardenolide differences and body size differences. Seiber et al. (1980) found no effect of ingested digitoxin (added to a controlled diet) upon the development time, food consumption, or body weight of fourth instar monarch larvae (see also Chapter V). However, if it is true that the negative correlations do reflect metabolic costs, then we must inquire why such costs should be paid by monarchs and not by queens. One possible explanation can be found in the different population structures of these two species. Monarchs are migratory, with most individuals in eastern North America flying to a few restricted areas in Mexico to overwinter (Urquhart and Urquhart, 1977). Mating occurs during the remigration back to North America each spring and most probably results in many matings between individuals which developed as larvae on different species of milkweed, some of which lack cardenolide (see Roeske et al., 1976). Many of these matings would therefore involve butterflies that had not been subjected to selection for allelochemic-tolerance. This would tend to recombine any evolving gene complexes for cost-free adaptation to allelochemicals. In contrast, queens are fundamentally non-migratory (Young, 1982), making only limited regional movements (Brower, 1961; Burns, 1983). This greater degree of philopatry should more often

result in inbred matings among individuals that fed, as larvae, on the same hostplant species. In areas where high-cardenolide plants predominate, this should facilitate the evolution of a more rapid, fine-tuned adaptation to hostplant allelochemicals.

Sexual Differences in Cardenolide Concentration

Previous studies of monarch butterflies have found that females have higher cardenolide concentrations than males (Brower and Glazier, 1975; Brower and Moffitt, 1974; Brower et al., 1972). This was again demonstrated here for the laboratory-reared monarchs. Brower and Glazier (1975) suggested that such a dimorphism might reflect relatively stronger selection for chemical defense in females which must spend considerable time exposed to potential enemies while searching for oviposition plants. However, males also incur certain risks in searching for females, and it is not clear how these risks to males are to be compared with risks to females when predicting differences in defensive strategies. In any event, such risks would presumably apply to the congeneric queen butterfly as well. The lack of sexual difference in cardenolide content of queens shown here therefore suggests that the "differential risk" hypothesis is not sufficient to explain the dimorphism previously observed in monarchs.

Another possible explanation is that female monarchs store more cardenolide than males as a means of providing for the defense of their eggs (Brower et al., 1982). Thomashaw (in Brower, in press)

reported that each egg of a female monarch reared on A. curassavica contained, on average, 0.97 ug of cardenolide. Since a female may lay from 100 (Erickson, 1973) to 400 (Urquhart, 1960) eggs, these could collectively contain as much as 97 to 388 ug of cardenolide. Adult monarchs reared on A. curassavica contain an average of 670 ug of cardenolide (Roeske et al., 1976). Thus, the amount placed by females into eggs constitutes a substantial proportion (14% to 58%) of this total. One might therefore expect cardenolides to be effective predator- or parasite-deterrents in monarch eggs but this has not yet been tested. The lack of sexual difference in cardenolides of queens may suggest that females of this species are not strongly selected to store these compounds for egg-defense, and leads to the prediction that they should allocate proportionally less cardenolide to eggs than do monarchs. Further work should be directed at this issue.

Interestingly, while laboratory-reared monarch (but, again, not queen) females did have significantly higher cardenolide concentrations than males, this was not observed in field-collections from the Miami area. This may suggest that some of the wild-caught females in the Miami sample had already laid some of their cardenolide-rich eggs, thereby reducing their (initially greater) cardenolide loads to a level similar to that of males. (This suggestion is consistent with Dixon et al.'s (1978) laboratory finding that female monarchs that had laid all of their eggs were less emetic when force-fed to pigeons than were freshly-eclosed females.) Since females would likely oviposit at varying rates, this should lead to a

greater variation in cardenolide concentration of females, relative to males. Indeed, females do tend to have higher variances than males for this trait (see Table 5; $F_{\text{max}} = 2.12$, $0.05 < p < 0.10$; Sokal and Rohlf, 1981).

Cardenolide Variability and its Implications

The great intra- and interspecific uniformity in qualitative cardenolide profiles of the Miami butterflies (see Fig. 4), suggests that a single hostplant species had been utilized by larvae of both species, but that there is individual variation in storage of certain compounds, especially those of highest R_f value. That these TLC profiles are virtually indistinguishable from those of monarchs reared in the laboratory on A. curassavica (Fig. 4 inset) strongly suggests that this was the hostplant species utilized by both danaid species in the Miami sample (cf. Fig. 5 for A. humistrata-reared butterflies). This represents the first practical application of the cardenolide "fingerprinting technique" (Brower et al., 1982) to identify the hostplants utilized, as larvae, by wild-caught danaid butterflies. It also demonstrates the potential of the technique as an aid in understanding the natural history and migration patterns of danaiids. Since the monarchs studied were collected in Miami in December and developed on A. curassavica (an introduced milkweed with a North American distribution restricted to southern states; Woodson, 1954), we may conclude that they were not merely migrant butterflies from

northern states that had "become trapped" in peninsular Florida en route to Mexico. Rather, this is strong evidence that monarchs breed in south Florida during the winter months.

A large percentage of each queen sample consisted of butterflies containing no measurable cardenolide (57% in Lake Istokpoga, 17% in Corkscrew Swamp, and 21% in Miami). Since there were no significant sex differences, such intrapopulational variability may instead reflect localized differences in hostplant species availability, intraspecific variation in plant cardenolide content (see, e.g., Nelson et al., 1981), or individual butterfly differences in cardenolide sequestration. Whatever its origin, such variability suggests the existence of a cardenolide-based palatability spectrum for queens, similar to that previously described for monarchs (Brower, 1969; Brower et al., 1968; Brower and Moffitt, 1974).

This result has important implications for understanding the southern viceroy's apparent switch from mimicking the monarch (as it does elsewhere in its range), to mimicking the queen in Florida (see Chapter I). If queens had been found to contain, on average, either more cardenolide than monarchs, or a different and potentially more potent (e.g., more emetic; Brower, 1969) set of cardenolides, then such a mimetic switch might be easily understood. However, the Miami queens clearly contained lower cardenolide concentrations and total amounts than did the sympatric monarchs. Since the two species had virtually identical cardenolide "fingerprints," it cannot be argued that queens stored a more noxious array of cardenolides than did

monarchs and were therefore more emetic even at lower concentrations. Thus, when fed on the same plants, monarchs and queens store the same cardenolides but monarchs concentrate these to a greater extent than do queens. This conclusion is further supported by laboratory rearings of the two species on Asclepias humistrata (Table 7 and Fig. 5). Moreover, Brower et al. (1975) have shown that, in order for an A. curassavica-reared monarch (sexes pooled) to be emetic to an 85 g blue jay on 50% of test trials, it must contain at least 76 ug of cardenolide. Of the Miami butterflies analyzed here, 85% of monarchs met this criterion of unpalatability, while only 30% of queens did so. It therefore seems that, at least with respect to cardenolides, queens are poorer models for viceroy mimicry than are monarchs.

Why then should the viceroy have abandoned its usual model in favor of the queen? Since monarchs are migratory, "pulsing" through Florida in large numbers only in the spring and fall (Urquhart and Urquhart, 1976; Brower, Malcolm and Cockrell, unpubl. data), while queens are more sedentary, the latter species would be spatiotemporally more "available" than monarchs to act as models in Florida. A theoretical model developed by Pough et al. (1973) showed that mildly noxious species could serve as suitable models for mimicry if they occurred in sufficient abundance. This may provide the explanation for the switch in viceroy mimicry. If resident, Florida monarch populations have been stable and predictable in their current locations for sufficient time, then reversals in the trend of mimicry might be expected, such that viceroys in those areas should tend to be

more "monarch-like" than those elsewhere in the state. A detailed geographic analysis of wing patterns is needed to test this prediction.

Alternatively, queens might, in fact, be superior models to monarchs, not because of their cardenolide content but, rather, due to sequestered pyrrolizidine alkaloids (PA's) that adults ingest from certain withering plants (Edgar, 1975; Edgar et al., 1979). While both sexes typically store these compounds as adults, male queens employ them further as precursors of their sex pheromone (Meinwald et al., 1969; Pliske and Eisner, 1969). Male and female monarchs are also somewhat attracted to PA sources and may store the alkaloids but males apparently do not use them as pheromone precursors (Edgar et al., 1976). While there is as yet no experimental verification of PA-based defense in danaid butterflies, K.S. Brown (unpublished manuscript) reports that certain neotropical spiders will release ithomiid butterflies from their webs unharmed if they contain PA's. The dependence of male queens (and not monarchs) on PA's for sexual competence suggests that, on average, queens may contain more of these compounds than monarchs, and therefore possibly serve as better models for viceroy mimicry. A comparative study of the PA concentrations of wild-caught monarchs and queens would shed further light on this intriguing problem.

Plant/Herbivore Coevolution

With respect to the issues of plant/herbivore coevolution presented in the Introduction, this study leads to the following provisional conclusions: (1) There is no evidence that the lower cardenolide concentrations sequestered by queens relative to monarchs reflects a poorer underlying tolerance for these allelochemicals. On the contrary, since only monarchs demonstrate significant negative correlations between body size and cardenolide concentration, it might be argued that monarchs are less adapted than queens for handling cardenolides. However, a causative connection between cardenolide sequestration and body size has not been established (see also Chapter V); (2) Despite quantitative differences between monarchs and queens in cardenolide storage, both species appear to sequester the same individual cardenolide compounds from their hostplants. Indeed, when reared on the milkweed A. humistrata, even dogbane tiger moths (Cycnia tenera; Arctiidae), also apocynad/asclepiad-specialists, produced the same characteristic TLC profile (Fig. 5) as did the polyphagous arctiid moth, Estigmene acraea (although only in trace amounts; Fig. 5). Moreover, Marty (1983) has shown that gut homogenates of both monarch and E. acraea larvae are capable of effecting a similar enzymatic transformation of one milkweed cardenolide, uscharidin, to two more polar metabolites (calactin and calotropin). Such similarity among taxonomically disparate Lepidoptera, whether oligo- or polyphagous, suggests that there may exist only a single qualitative

route of cardenolide processing in this insect order but that further evolution may involve a quantitative increase in tissue cardenolide concentration in accordance with the defensive requirements of each species. However, the basic biochemical processes shared by all these species may represent a common preadaptation to feeding on milkweeds or other cardenolide-containing plants.

In summary, this chapter has demonstrated that although monarch butterflies are the most thoroughly-studied danaids, they may not be representative of their family in respect to adaptation to hostplant allelochemicals. A better understanding of danaid/milkweed relationships will therefore depend upon obtaining a broader comparative data base to document differential species responses to ingested or sequestered cardenolide. Here it was shown that although monarchs and queens sequester the same qualitative set of cardenolides from milkweed plants, monarchs concentrate these in their tissues to a greater extent than do queens. Furthermore, adult monarchs exhibit significant negative correlations between the concentration of sequestered cardenolide and both body size and weight, whereas queens show no such correlations. Although this may suggest a metabolic cost paid by monarchs and not by queens (and perhaps attributable to different life-history characteristics; see above), there is, as yet, no direct evidence of a causal relationship between cardenolide differences and body size or weight differences. This question will be explored more directly in Chapter V, following a consideration in Chapter IV of cardenolide sequestration in a third milkweed-specializing insect, the dogbane tiger moth (Cycnia tenera). This

species was previously reported not to sequester hostplant cardenolides (Rothschild et al., 1970). This raises the interesting question of whether, even among specialists, some species do not sequester cardenolides because they are more sensitive to these compounds than are sequestering species. If so, then species such as C. tenera might represent an intermediate stage in the evolution of allelochemic-adaptation.

CHAPTER IV

CARDENOLIDE SEQUESTRATION BY THE DOGBANE TIGER MOTH (CYCINIA TENERA; ARCTIIDAE)*

Introduction

Immature stages of monarch butterflies (Danaus plexippus L.; Parsons, 1965; Reichstein et al., 1968; Brower, 1969) and milkweed bugs (Hemiptera: Lygaeidae; e.g. Scudder and Duffey, 1972; Isman et al., 1977a,b) are well-known for their ability to sequester plant defense chemicals known as cardenolides (cardiac glycosides) from their milkweed hostplants (Asclepiadaceae). Moreover, laboratory studies have shown that these stored chemicals protect the insects from some vertebrate predators (Brower and Brower, 1964; Brower, 1969; Rothschild and Kellett, 1972). Several other insect species feed at least occasionally on milkweed plants (Wilbur, 1976; Price and Willson, 1979), and some of these have been assayed for the presence or absence of cardenolides (e.g., Duffey and Scudder, 1972; Rothschild and Reichstein, 1976). However, for relatively few species has it

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been experimentally demonstrated that the cardenolides found in wild and/or lab-reared insects are indeed derived from the hostplants (allochthonous) rather than synthesized by the animals de novo (autochthonous), as occurs, for example, in some chrysomelid beetles (Pasteels and Daloze, 1977; Daloze and Pasteels, 1979). One may distinguish these two possibilities by simultaneous rearings of insects on cardenolide-rich and cardenolide-poor diets (e.g., Brower et al., 1967; Rothschild et al., 1978). Differences in the cardenolide contents of insects reared on such foods may then be attributed to these dietary differences. This approach was taken in the present study of the dogbane tiger moth, Cycnia tenera Huebner (Arctiidae), a species previously reported not to sequester hostplant cardenolides (Rothschild et al., 1970). Since a related species (C. inopinatus), which is also a specialist feeder on asclepiadaceous and apocynaceous plants (Tietz, 1972; Nishio, 1980), has since been found to store these chemicals (Nishio, 1980), a reevaluation of C. tenera seemed desirable. Among other arctiids, two species (Arctia caja and Euchaetias antica) are known to sequester hostplant cardenolides (see Rothschild and Reichstein, 1976). While three others (Euchaetias egle, Digama aganais, D. sinuosa) were reportedly devoid of cardenolides, it remains unclear whether this was due to storage inability or perhaps to feeding on milkweeds low, or lacking in these compounds. Sufficient intraspecific and interspecific diversity of milkweed cardenolide contents exists (e.g., Roeske et al., 1976; Nelson et al., 1981; Seiber et al., in press), so that it is possible

for an herbivore to be a milkweed specialist without being a cardenolide specialist (see also Rothschild et al., 1970). However, if C. tenera are found to sequester cardenolides then the hypothesis that they pay metabolic costs of doing so may be evaluated by searching for correlations between the concentration of sequestered cardenolide and body weight (see Chapter III for rationale).

Cycnia tenera females lay clutches of 50 to 100 eggs (personal observations), and larvae feed gregariously on their hostplants (we have frequently seen groups of 5-7 larvae on a single A. humistrata leaf). If the female has mated only once prior to oviposition, these larvae will be full-siblings whereas, if she has mated more than once, they will be at least half-siblings. This high degree of relatedness permits the evolution of unpalatability and aposematism via kin selection (Hamilton, 1964; Harvey et al., 1982; Brower, in press). Thus, if cardenolides are, in fact, sequestered by the larvae and retained into adulthood (both stages being aposematic; see Fig. 6), then they may contribute importantly to the chemical defense and evolution of this species.

Methods

In north-central Florida, Cycnia tenera larvae are commonly found on Asclepias humistrata, a milkweed species having a relatively high cardenolide concentration in its leaves (Nishio, 1980; cf. Roeske et

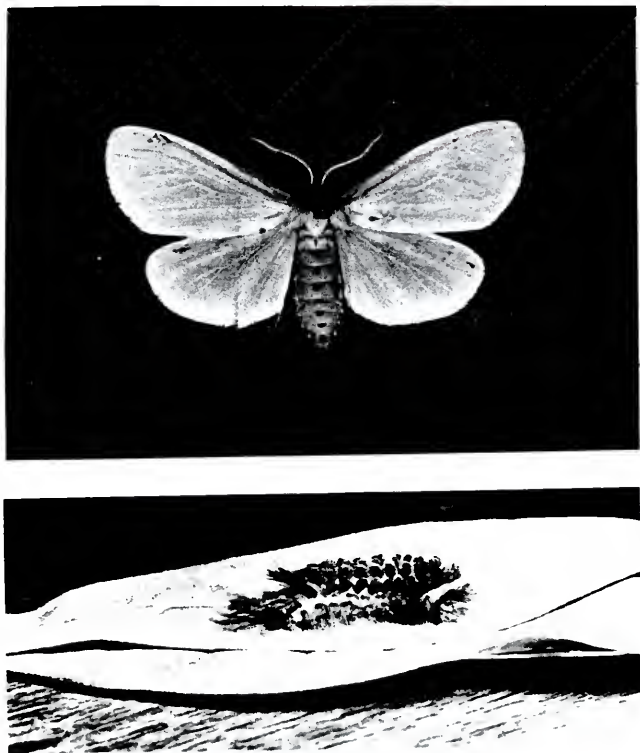


Fig. 6. An adult male Cycnia tenera and a late-instar larva. The taxonomy of this genus has been disputed. Forbes (1960) considered C. tenera as distinct from C. inopinatus, while Kimball (1965) merged both species under the latter name. More recent treatments (e.g., Hodges, in press) maintain separate status for the two species. Voucher specimens of larvae and adults used in this study have been deposited in the Florida State Collection of Arthropods, Doyle Conner Building, Gainesville, FL. (Photos by L.P. Brower)

(Source: Cohen and Brower, 1983)

al., 1976; see also Chapters I and II). A clutch of 54 C. tenera eggs was collected on 12 May 1981 from an A. humistrata plant growing wild approximately 3 Km W of Gainesville (Alachua County), FL. The eggs were brought to the laboratory and, upon hatching, each larva was placed in an individual 250 cc closed (vented) plastic container and reared to eclosion at $23 \pm 1^{\circ}\text{C}$. Of these, 7 were reared through to adulthood on a total diet of A. humistrata leaves, while 12 fed only on A. tuberosa, a local species known to contain only very slight amounts of cardenolide (Chapter I; also Roeske et al., 1976). All adult moths were killed by freezing between 12 and 24 hours after eclosion and remained frozen until chemical analysis in March, 1982.

Prior to analysis, the moths were dried at 60°C for 16h in a forced-draft oven. Dry weights were determined to the nearest 0.1 mg using a Mettler AK 160 electronic balance. Fat was then removed from each insect by petroleum ether extraction of the dried material for 30 min in a 35°C shaker bath (methods in Walford and Brower, in preparation). Fat extraction removes only negligible amounts of milkweed cardenolide from the insect material (C. Nelson and L.P. Brower, unpublished data; see also Nishio, 1980).

Cardenolides were extracted from the fat-free material and concentrations determined (as microgram equivalents of digitoxin) using spectrophotometric procedures described in Brower et al. (1972, 1975). A lead-acetate clean-up procedure (Brower et al., 1982) was then used to remove interfering pigments and other noncardenolide compounds from the extract remaining after spectroassay. Thin-layer

chromatography (TLC), employing an ethyl acetate:methanol solvent system (97:3 by volume; see Brower et al., 1982), was used to visualize the cardenolides present in each hostplant species and in two randomly selected moths that had developed on each species.

Results

The mean dry and lean weights, cardenolide concentrations and total cardenolide contents of C. tenera adults reared on A. humistrata and A. tuberosa are shown in Table 9. Two-way analyses of variance demonstrate that females reach heavier dry weights than do males, regardless of foodplant eaten ($F=32.5$, $df=1,15$, $p<0.001$), but that both sexes are heavier when reared on A. humistrata than on A. tuberosa ($F=40.9$, $df=1,15$, $p<0.001$). The interaction of sex and foodplant was not significant ($p>0.50$). The results for lean weights are similar, with females heavier than males ($F=16.2$, $df=1,15$, $p<0.005$) and both sexes tending to be heavier on A. humistrata than on A. tuberosa ($F=5.6$, $df=1,15$, $0.1>p>.05$). Females contain slightly more fat than do males ($F=4.6$, $df=1,15$, $0.1>p>.05$) and both sexes store more fat when fed A. humistrata than when fed A. tuberosa ($F=21.2$, $df=1,15$, $p<0.001$). Overall, the fat content of the adult moths constitutes from 45 to 60 per cent of the total dry weight.

Table 9. Means ($\bar{X}$) and standard deviations (SD) for the weights and cardenolide contents of adult Cycnia tenera reared on the milkweeds, Asclepias humistrata and A. tuberosa.

HOSTPLANT	SEX	N	DRY WEIGHT (mg)		LEAN WEIGHT (mg)		WEIGHT OF EXTRACTED FAT (mg)		CARDENOLIDE CONCENTRATION (ug/0.1g)		TOTAL CARDENOLIDE PER MOTH (ug)	
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
<u>Asclepias humistrata</u>	M	3	30.8	5.1	12.3	2.4	18.5	2.8	759	290	90	29
	F	4	44.4	4.4	20.8	6.4	23.6	4.0	645	175	135	62
<u>Asclepias tuberosa</u>	M	8	17.2	3.8	7.5	2.4	9.7	2.7	98	90	8	8
	F	4	29.2	5.9	15.8	5.6	13.4	7.0	53	37	8	7

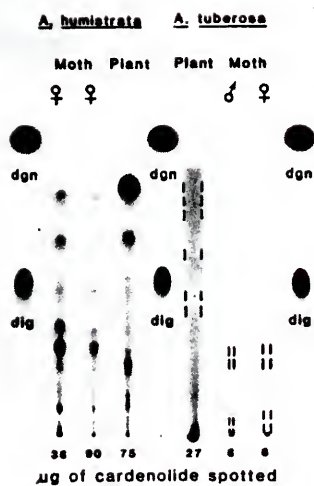
Source: Cohen and Brower, 1983

Clearly, both sexes sequestered higher concentrations and total amounts of cardenolide from A. humistrata than from A. tuberosa ($F=76.4$ for concentration; $F=50.0$ for total amount; $df=1,15$ and $p<0.0001$ for both tests). However, the sexes did not differ significantly either in concentration ($F=1.2$, $df=1,15$, $p>0.2$) or in total cardenolide content ($F=2.4$, $df=1,15$, $p>0.2$). No significant interaction effects were identified ($p>0.2$). When both sexes are considered together, moths reared on A. humistrata had, on average, 10 times higher concentrations, and contained 15 times more total cardenolide, than did moths reared on A. tuberosa.

Spearman rank order correlations demonstrate no significant relationship between the concentration of sequestered cardenolide and either dry weight ($r=-0.28$; $P>0.50$) or lean weight ($r=-0.43$; $P>0.30$). This analysis was limited to those moths reared on A. humistrata ($n=7$; sexes pooled) in order not to confound cardenolide-differences with other plant species differences (e.g., in nutritional quality; see Discussion).

Thin-layer chromatography (Fig. 7) clearly confirms that sequestration of hostplant cardenolides occurred, since individuals reared on A. humistrata contain virtually every cardenolide visualized in their hostplants. However, the chromatogram of A. tuberosa, a very low-cardenolide species, consists only of a faint band ranging from approximately $R_f = 0.1-0.5$. Much of this band is pink (i.e., noncardenolide interference) rather than blue (cardenolide) and it is possible that these interfering compounds may have contributed to the

Fig. 7. TLC profiles of four adult Cycnia tenera reared on either Asclepias humistrata (2♀♀) or A. tuberosa (1♂, 1♀). For reference, a mixture of commercially procured digitoxin (dig; Rf = 0.31) and digitoxigenin (dgn; Rf = 0.60) was spotted in three channels. The amounts of cardenolide (ug equivalent to digitoxin) spotted in each channel are shown at the bottom of the plate. (These are calculated prior to lead-acetate clean-up, a procedure which sometimes reduces the amount actually spotted. Thus, the female in channel 3 has a lighter trace than that in channel 2, presumably having lost disproportionately more cardenolide during clean-up.) For A. humistrata there is a very close correspondence between the cardenolides present in the leaf tissue and those in the moths reared thereupon. However, A. tuberosa has a very low cardenolide concentration and only faint spots are produced from the leaf and moth extracts. In the original color prints, all cardenolides appear as blue spots. However, much of the A. tuberosa leaf and moth chromatographs appears pink in the color original, except for faint blue cardenolide regions indicated by broken lines. No such interference occurs in the chromatographs of A. humistrata, the moths reared thereupon, and the commercial standards. (Source: Cohen and Brower, 1983)



absorbances recorded for the moths reared on this species, thereby overestimating cardenolide concentrations. If this is so, then the true difference in cardenolide contents between moths reared on the two hostplants may be even greater than reported above.

Discussion

These data demonstrate the sequestration of hostplant cardenolides by Cycnia tenera larvae and the subsequent retention of these chemicals into adulthood. Individuals reared on Asclepias humistrata, a cardenolide-rich milkweed species, attained much higher concentrations and total amounts of cardenolide than did those reared on a cardenolide-poor diet of A. tuberosa. Moreover, TLC analysis demonstrates a close correspondence between the individual cardenolides of A. humistrata and those of the moths reared thereupon. If all of the cardenolide present in C. tenera were of autochthonous origin, moths reared on both milkweed species would be expected to contain similar amounts and kinds of cardenolide. Clearly, they do not. However, the possibility that the small amount of cardenolide present in A. tuberosa-reared moths may, at least in part, be of autochthonous origin cannot be excluded.

Both sexes reached higher fat and lean weights when reared on the higher cardenolide species, A. humistrata. Similar results for Danaus plexippus (Erickson, 1973), D. chrysippus (Smith, 1978), and

Oncopeltus fasciatus (Isman, 1977; Chaplin and Chaplin, 1981) have been interpreted as evidence against a metabolic cost of handling hostplant cardenolides (see also Blum, 1981). However, this inference is problematical because milkweed species differ in many ways other than in cardenolide content (e.g., nitrogen and water content, Erickson, 1973; leaf shape, growth form, texture and pubescence, Woodson, 1954). Such differences contribute to the overall suitability and quality of these plants as insect food (Scriber and Slansky, 1981). Asclepias tuberosa is a relatively poor food source due to low nitrogen and water content (Erickson, 1973) and is infrequently used by herbivores in north-central Florida (Cohen and Brower, unpublished observations), or elsewhere (e.g., Wilbur, 1976; Price and Willson, 1979). It is to be expected that such poor foodplant species will be under weaker selection for antiherbivore adaptations than will species of higher nutritive value, and might therefore have relatively lower cardenolide contents. Hence, it is possible that herbivores develop more successfully on relatively-high cardenolide species, such as A. humistrata, not because there is no cost of handling these chemicals, but because the higher food quality is great enough to offset any such costs that might exist.

This complication explains why correlations between insect cardenolide concentration and body weight were sought only for those individuals reared on A. humistrata. Clearly, had the A. tuberosa-reared moths been included in the analysis, a positive correlation would result. However, this would most likely be due to the

nutritional differences between the two plant species, rather than their allelochemic differences. That the A. humistrata-reared moths exhibited no significant correlations between cardenolide concentration and body weight provides further (indirect) evidence that milkweed-specializing lepidopterans pay little, if any, cost of feeding on cardenolides.

It is interesting that the extracted fat from C. tenera adults may constitute from 45 to 60% of the total dry weight (Table 9). This is sharply higher than the 5 to 20% common for freshly eclosed danaid butterflies (Beall, 1948) and is similar to the levels found in migrating monarch butterflies just prior to overwintering in Mexico (Walford and Brower, in prep.). Moths of a related arctiid genus, Euchaetias, do not feed as adults (Forbes, 1960; Schroeder, 1977) and lifetime energy stores must be accumulated by the larvae prior to pupation. However, unlike Euchaetias, adult Cynia have well-developed probosces (Forbes, 1960), and presumably feed. Thus their apparently high fat storage remains enigmatic. While this result is not directly relevant to the issue of cardenolide-defense, it does raise an intriguing question and it should prove interesting to compare larval fat storage between those arctiid species which have a feeding adult stage and those which do not.

The results on cardenolide sequestration are in contrast to those of Rothschild et al. (1970), in which no cardenolides were found in C. tenera reared on Asclepias syriaca. That plant species, however, is extremely variable in cardenolide content (see Roeske et al., 1976),

and certain strains are reportedly devoid of cardenolides (Rothschild et al., 1975). It is thus possible that the particular plants fed to the larvae in the experiments of these authors were of insufficient cardenolide content for larval sequestration to occur. Alternatively, intraspecific geographic differences in sequestration abilities in the moths may be indicated. Such differences have been hypothesized for the various geographic races of the African queen butterfly, Danaus chrysippus (Rothschild, et al., 1975) but have not yet been definitively established for any species. A third possibility is that C. tenera is not adapted to sequester the particular array of cardenolides found in A. syriaca. Indeed, of 6 milkweed species studied by Price and Willson (1979) in central Illinois, A. syriaca was one of only two never utilized by C. tenera as a larval foodplant. However, there is no evidence that this apparent rejection was determined in any way by the cardenolides of A. syriaca.

Cycnia tenera adults are conspicuously colored, with off-white wings and black-spotted, yellow abdomens (Fig. 6). The bitter-tasting cardenolides they contain probably impart to them a noxious quality, as is true in the monarch butterfly (Brower, 1969; Brower and Moffitt, 1974). Moreover, they produce both audible and ultrasonic sounds (Fullard, 1977) which may serve to warn bats and other potential predators of the unpalatability of the moths, thus serving an aposematic function (e.g., Dunning and Roeder, 1965).

Larvae, too, are aposematic, with bright orange bodies and contrastingly-dark tufts of setae along the dorsum (Fig. 6).

Interestingly, the setae of a closely related species, C. inopinatus, are virtually devoid of cardenolides, while the underlying larval cuticle is rich in these compounds (Nishio, 1980). This suggests the potential operation of three separate lines of defense in larval Cycnia. The setae may have a sensory function, permitting the larvae to recognize the approach of a predator or parasite. Larvae respond to tactile stimulation by dropping to the ground and curling the body (unpublished observations). This behavior has the effect of exposing the dorsal setae maximally. Should the larva nevertheless be found by a vertebrate predator, a second, mechanical line of defense may come into play: naive predators may attempt to eat a larva, but release it unharmed when the mouth is irritated by the hairs. The setae may then become an aposematic signal, preventing further attacks by experienced predators. The evolution of such sensory and mechanical defense is not problematic since larvae having such setae would presumably be more likely than those lacking them to survive an attack. If, however, these defenses should fail, the cardenolides present in the larval tissues would provide an unpalatable, and possibly emetic, experience (see, e.g., Brower, 1969), leading to later avoidance of further larvae encountered. Indeed, it is possible that the setae, once ingested, may irritate the gastro-intestinal lining (Bisset et al., 1960; Frazer, 1965) and thereby facilitate absorption of cardenolides, i.e., an interaction of mechanical and chemical defenses! The larva would die in the process, however, and thus its genes for cardenolide sequestration would not be propagated in the

population. Kin-selection (see Hamilton, 1964) would be required for the evolution of such chemical defense and seems plausible in this species, since eggs are laid in clutches and the larvae feed gregariously on their hostplants. Thus, kin-groups of at least half-siblings, and possibly full-siblings, feed together on the same plant. If a predator were to sample and kill one or a few of these aposematic larvae before learning to avoid them, the remaining siblings would be spared and shared genes both for unpalatability and aposematism would continue to spread within the population.

My work on cardenolides in C. tenera suggests that these compounds may provide at least a partial basis for an underlying unpalatability of both larvae and adults. However, this does not preclude the possibility of other chemical defenses. Parsons and Rothschild (in Rothschild et al., 1970) have noted the presence of histamine-like and/or acetylcholine-like substances in C. tenera, although the manner in which these compounds function in nature remains largely unexplored. Moreover, several species of arctiid moths (including Cynia mendica; Rothschild, 1973) are known to sequester pyrrolizidine alkaloids (PA's) from larval hostplants (although these have not yet been identified from Asclepiadaceae) or, for those species with feeding adult stages, from decomposing leaves (Rothschild et al., 1979). Finally, it is possible that other noxious chemicals in these moths may be of autochthonous origin, rather than derived from plant sources (Rothschild et al., 1970, 1979). The relative contributions and possible interactions of cardenolides, biogenic amines, PA's,

and/or other noxious substances in the defensive strategies of insects can only be determined through controlled-rearing schemes followed by ecologically relevant predation studies.

In this chapter, I have confirmed that, contrary to previous reports, dogbane tiger moths do sequester milkweed cardenolides. Like the queen butterfly (Danaus gilippus) discussed in Chapter III, but unlike monarchs, these moths, when reared on Asclepias humistrata, exhibited no correlation between sequestered cardenolide concentration and body weight. This argues against the hypothesis that a metabolic cost is paid by specialists. Moreover, those individuals reared on a high-cardenolide milkweed species (A. humistrata) achieved significantly higher body weights than did those reared on a very low-cardenolide species (A. tuberosa). While several other workers have interpreted such results in other insect species as evidence against the metabolic cost hypothesis, such interpretations confound cardenolide differences among milkweed species with other nutritionally relevant differences. These confounding factors were eliminated in the experiments described in the next chapter, in which purified cardenolides were added to otherwise uniform diets.

CHAPTER V

THE EFFECTS OF INGESTED CARDENOLIDE UPON FOOD CONSUMPTION AND GROWTH OF SPECIALIST AND GENERALIST LEPIDOPTERAN LARVAE

Introduction

The theory of plant/herbivore coevolution (Ehrlich and Raven, 1964) has played a major role in organizing a very diverse literature on the defensive strategies of plants against their enemies, as well as enemy counter-offenses (see, e.g., Feeny, 1976; Rosenthal and Janzen, 1979; Futuyma and Slatkin, 1983). In essence, plants are viewed as defended by chemical and other means (e.g., mechanical and phenological) against a broad array of potential attackers. In the course of evolution, a few mutant individual herbivores may find themselves able to circumvent the plant's defensive barrier and enter a new adaptive feeding zone, relatively free from interspecific competition. This, in turn, selects for subsequent counter-defenses by the plant population, counter-offenses by the herbivore population, and so on.

It has been widely held (e.g., Brower and Huberth, 1977; Dixon et al., 1978; Harborne, 1983) that the classic example of coevolution

between insect herbivores and their larval foodplants is that of the monarch butterfly (Danaus plexippus L; Danaidae), which develops on milkweed plants (Asclepiadaceae). Many milkweed species contain cardenolides (cardiac glycosides), chemicals which are potent vertebrate heart poisons. However, there is no evidence (nor, indeed, has it ever been tested) that ingested cardenolides provide any kind of barrier to herbivory by non-adapted insects. Clearly, such a barrier must be shown to exist before it becomes plausible to think of adapted species, such as monarchs, as having "breached" a chemical defense during the course of coevolution.

Here I test the effects of ingested cardenolide upon the consumption rates, growth rates, and food utilization efficiencies of three lepidopteran herbivores: a) a milkweed "specialist," the monarch butterfly, Danaus plexippus L. (Danaidae); b) a highly polyphagous species or "generalist," the fall armyworm, Spodoptera frugiperda (J.E. Smith) (Noctuidae), and c) a species specializing on a plant family lacking cardenolides (i.e., a "negative-specialist"), the velvetbean caterpillar, Anticarsia gemmatilis Hübner (Noctuidae). A similar three-way comparison by Blau et al. (1978), in which sinigrin, the defensive compound of cruciferous plants, was added to controlled diets, demonstrated no significant negative effects upon a crucifer-specialist, moderate effects upon a generalist, and severe effects upon a negative-specialist. Similar results were predicted in the present study because generalists, which consume plants from many diverse families, should be selected for an optimal, broad-scale

detoxification ability, but not to handle any one class of compounds as effectively as would a specialist on those compounds (Feeny, 1975; Rhoades and Gates, 1976). Moreover, a negative-specialist might be most severely affected since, although it has a fine-tuned detoxification ability, this is directed at a wholly different class of compounds.

Methods

Three separate experiments were conducted, each with one of three lepidopteran species (details provided below), but all utilizing the same basic experimental approach. This involved the addition of varying amounts of cardenolide (digitoxin, Sigma Chemical Co.) to otherwise standardized diets. Gravimetric analyses (Waldbauer, 1968) were performed for the entire duration of the penultimate instar in each case and yielded the following performance indices (all weights are dry):

- Relative Growth Rate (RGR): This expresses the amount of weight gained per unit mean body weight, normalized to a one-day time period. An RGR of 1.0 thus indicates that an animal adds 100% of its (dry) body weight in new growth each day. A decrease in RGR with increasing cardenolide in the diet could result either from a decrease in consumption rate (RCR) or from a decrease in the efficiency with which ingested food is converted into new biomass (ECI), or both.

- Relative Consumption Rate (RCR): This expresses the weight of food eaten as a proportion of the mean body weight, normalized to a one-day time period. Thus, an RCR of 1.0 indicates that an individual consumes its own (dry) weight in food each day. A decrease in RCR with increasing cardenolide in the diet would indicate a behavioral feeding deterrent effect of these chemicals.

- Efficiency of Conversion of Ingested Matter (ECI): This expresses the weight gained as a percentage of the weight of food consumed. Thus, an ECI of 50% indicates that half of the food eaten is converted into new body growth. A decrease in ECI with increasing cardenolide would suggest a "cost" of handling these allelochemicals because additional time and energy expenditures, in terms of feeding behavior, would be required to support the same amount of growth.

Larvae were housed individually in 12 ml stoppered glass vials (Seiber et al., 1980) and maintained in the laboratory between 23 and 25°C and ambient light conditions. Gravimetric testing began upon molting into the penultimate instar and ended upon molting into the final instar. All determinations of cardenolide concentrations were made by standard spectrophotometric procedures (Brower et al., 1972, 1975). Details specific to each experiment are as follows:

Experiment 1 - The Specialist: Monarch Butterfly

Larvae used were the progeny of a single female, collected as a larva in Gainesville, Alachua Co., FL, reared to maturity and mated in the laboratory to a similarly-reared male. All larvae were fed from hatching on an exclusive diet of Asclepias tuberosa leaves (root-stock obtained from Gurney Seed & Nursery Co., Yankton, S. Dakota). Leaves of this plant species contain very low to negligible amounts of cardenolide (see Chapter I; also Roeske et al., 1976). Upon molting into the penultimate (fourth) instar, 23 larvae were randomly assigned to treatment groups and given a weighed surfeit of leaves of one of four kinds:

a) Undipped Control: These were leaves identical to those fed to the earlier instars. Mean cardenolide concentration was 0.03% by dry weight (S.D.=0.02%; N=3).

b) Water-Dipped Control: Leaves briefly dipped into distilled water and allowed to air-dry before feeding. Mean cardenolide concentration 0.03% (S.D.=0.02%; N=3).

c) High Cardenolide: Leaves briefly dipped into an (distilled) aqueous suspension of digitoxin. Mean cardenolide concentration 1.6% (S.D.=0.28%; N=3).

d) Very High Cardenolide: Leaves briefly dipped, as above, to produce a mean concentration of 2.6% (S.D.=0.55%; N=3).

Naturally occurring cardenolide concentrations in milkweeds rarely exceed 1.0% (Roeske et al., 1976). As both concentrations used in this experiment were beyond this limit, they should be sufficient to reveal any negative effects which might accrue to the larvae.

Experiment 2 - The Generalist: Fall Armyworm

Populations of this species are known to feed on plants from many diverse families (e.g., Tietz, 1972). Eggs were collected from stock of the U.S.D.A. Insects Attractants, Behavior, and Basic Biology Research Laboratory (Gainesville, FL) and larvae fed ad lib from hatching on one of four diets:

a) Control: This consisted of cardenolide-free artificial diet, as described by Leppla et al. (in press).

b) Low Cardenolide: Artificial diet into which powdered digitoxin was mixed to produce a mean allelochemic concentration of 0.17% by dry weight (S.D.=0.02%; N=3).

c) Medium Cardenolide: Artificial diet brought to a mean of 0.56% cardenolide (S.D.=0.09%; N=3).

d) High Cardenolide: Artificial diet brought to a mean of 1.20% cardenolide (S.D.=0.33%; N=3).

In total, 49 larvae were tested gravimetrically upon molting into the penultimate (fifth) instar. Additional larvae were maintained on the various diets for up to 21 days in order to study survival (N=167) and development time (N=145).

Experiment 3 - The Negative Specialist: Velvetbean Caterpillar

This species feeds only on plants in the family Leguminosae (Tietz, 1972), none of which is known to contain cardenolides. Eggs were collected from U.S.D.A. stock (as above for fall armyworms) and larvae fed ad lib from hatching on one of five diets:

a) Control: Cardenolide-free artificial diet (Leppa et al., in press).

b) Aerosol Control: Artificial diet to which was added a slight amount of the wetting agent, Aerosol OT (Fisher Scientific Co.) to improve the solubility and dispersion of digitoxin in the diet (see, e.g., Nielsen, 1978). The total concentration of the wetting agent in the diet was 0.008% by volume.

c) Low Cardenolide: Artificial diet, Aerosol (as above), and sufficient digitoxin to produce a mean cardenolide concentration of 0.21% by dry weight (S.D.=0.03%; N=4).

d) Medium Cardenolide: Artificial diet and Aerosol, brought to a mean of 0.53% cardenolide (S.D.=0.06%; N=4).

e) High Cardenolide: Artificial diet and Aerosol, brought to a mean of 0.97% cardenolide (S.D.=0.10%; N=4).

In total, 66 larvae were tested gravimetrically upon molting into the penultimate (fifth) instar. Additional larvae were maintained on the various diets for 17 days in order to study survival (N=150) and development time (N=113).

Due to heteroscedasticity and non-normality of data, RGR, RCR, and ECI values for all three species were tested for significance using Kruskal-Wallis nonparametric analyses of variance (approximate chi-squared distribution), as provided by Helwig and Council (1979).

Results

Monarch Butterflies

Values of relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested matter (ECI) are shown

Table 10. Relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested matter (ECI) for fourth instar monarch butterfly larvae reared on four Asclepias tuberosa-based diets incorporating different cardenolide concentrations. Data are $\bar{X} \pm SD$ with sample sizes in parentheses.

<u>TREATMENT</u>	<u>% CAR-DENOLIDE</u>	<u>RGR</u> <u>(mg/mg/d)</u>	<u>RCR</u> <u>(mg/mg/d)</u>	<u>ECI</u> <u>(%)</u>
Undipped	0.03	0.18 ± 0.15 (7)	1.24 ± 0.53 (7)	15.11 ± 4.81 (6)
Water	0.03	0.22 ± 0.16 (5)	1.36 ± 0.68 (5)	18.37 ± 1.06 (3)
High Car-denolide	1.6	0.24 ± 0.15 (5)	1.70 ± 0.64 (5)	15.52 ± 2.15 (4)
Very High Cardenolide	2.6	0.26 ± 0.09 (6)	1.72 ± 0.45 (6)	15.20 ± 4.44 (6)
Kruskal-Wallis χ^2		1.85	3.54	1.53
df		3	3	3
P		0.60	0.32	0.68

in Table 10. Kruskal-Wallis analyses of variance demonstrate no significant heterogeneity among treatment groups for any of these variables, even at cardenolide concentrations well above those encountered in nature. There is, however, a slight (nonsignificant) trend toward higher consumption and growth rates as the cardenolide concentration increases. No such trend is evident for the conversion efficiency.

Fall Armyworms

RGR, RCR, and ECI values are shown in Table 11. RGR was unaffected at low cardenolide concentrations but was significantly depressed at medium and high dosages. This decline can be traced to a significant trend toward lower RCR as cardenolide level increased: when pooled, the control and low-cardenolide groups had significantly greater RCR values than did the pooled medium- and high-cardenolide groups (Wilcoxon 2-sample test, $p < 0.002$). Moreover, as allelochemic concentration increased, there was a significant general decline in ECI, which also contributed to the lower RGR values.

Additional data on the length of time required to develop from hatching to the start of the sixth instar are shown in Table 12A. This period was significantly lengthened as cardenolide concentration increased. Measurements of body weight at the start of the fifth instar (Table 12B) show that this prolongation of the development time enabled the larvae to maintain an approximate "target weight," despite

Table 11. Relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested matter (ECI) for fifth instar fall armyworm larvae reared on artificial diets incorporating varying amounts of cardenolide. Data are $\bar{X} \pm SD$ with sample sizes in parentheses. Asterisks indicate values that are significantly different from control at the (*) 0.05 or (**) 0.01 level by Kruskal-Wallis chi-square approximation (Helwig and Council, 1979).

<u>TREATMENT</u>	<u>% CAR-DENOLIDE</u>	<u>RGR (mg/mg/d)</u>	<u>RCR (mg/mg/d)</u>	<u>ECI (%)</u>
Control	0	0.52 $\pm$ 0.08 (11)	1.65 $\pm$ 0.35 (11)	32.29 $\pm$ 4.77 (11)
Low Car-denolide	0.17	0.52 $\pm$ 0.03 (11)	1.75 $\pm$ 0.15 (11)	30.06 $\pm$ 2.58* (11)
Medium Car-denolide	0.56	0.43 $\pm$ 0.11* (16)	1.43 $\pm$ 0.41 (16)	30.62 $\pm$ 3.67* (16)
High Car-denolide	1.20	0.44 $\pm$ 0.07** (10)	1.53 $\pm$ 0.22 (10)	28.84 $\pm$ 3.32* (10)
Kruskal-Wallis χ^2		19.39	14.06	9.62
df		3	3	3
P		0.0002	0.003	0.02

Table 12. Additional data for fall armyworms reared on artificial diets incorporating varying amounts of cardenolide. (A) Duration of the larval period from hatching until the start of the sixth instar. (B) Estimated dry weights of newly-molted, fifth instars. The estimation is arrived at by multiplying the fresh weight by the dry weight:fresh weight ratio determined on a similarly reared sample of larvae. (C) 2X4 contingency table showing the numbers of larvae dead or alive after 21 days feeding.

	TREATMENT			
	<u>CARDENOLIDE</u>			
	<u>CONTROL</u>	<u>LOW</u>	<u>MEDIUM</u>	<u>HIGH</u>
	(0.17%)	(0.56%)	(1.20%)	
(A) Larval Period (Hatching-VI)				
Median Days	10	11	12	12
Range	9-13	9-16	10-20	11-20
N	32	38	43	32
Kruskal-Wallis $\chi^2=42.92$, $df=3$, $P=0.0001$				
(B) Estimated Dry Weight (V)				
$\bar{X}$	4.43	4.18	4.19	3.69
S.D.	0.74	1.00	1.26	1.21
N	23	21	20	18
One-way ANOVA $F_{3,78}=1.70$, $P=0.17$				
(C) Survival After 21 Days				
No. Alive	27	37	39	32
No. Dead	8	5	7	12
$\chi^2 = 4.5$, $df = 3$, $P > 0.10$				

a tendency to be lighter when fed higher cardenolide diets. There was no significant dose-dependent mortality (Table 12C).

Velvetbean Caterpillars

RGR, RCR, and ECI values are shown in Table 13. Kruskal-Wallis tests demonstrate no significant heterogeneity among treatment groups for any of these variables. However, the length of time required to reach the sixth instar increased markedly when cardenolide was added to the diet (Table 14A). Despite this increase in development time, estimated dry weights at the start of the gravimetric experiment declined significantly with cardenolide (Table 14B), reflecting the cumulative effect of the allelochemic upon growth of the first four instars. Direct mortality also increased with cardenolide concentration (Table 14C). Nearly 82% (9/11) of these mortalities occurred in the first two instars.

Discussion

Previous studies of the effects of ingested cardenolides on herbivores have focused entirely on adapted, milkweed-specializing species (Danaus plexippus: Erickson, 1973; Dixon et al., 1978; Seiber et al., 1980; Danaus chrysippus: Smith, 1978; Oncopeltus fasciatus

Table 13. Relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested matter (ECI) for fifth instar velvetbean caterpillars reared on artificial diets incorporating varying amounts of cardenolide. Data are $\bar{X} \pm SD$ with sample sizes in parentheses.

<u>TREATMENT</u>	<u>% CAR-DENOLIDE</u>	<u>RGR (mg/mg/d)</u>	<u>RCR (mg/mg/d)</u>	<u>ECI (%)</u>
Control	0	0.39 ± 0.04 (13)	1.20 ± 0.10 (13)	32.78 ± 2.69 (13)
Aerosol Control	0	0.39 ± 0.03 (13)	1.26 ± 0.12 (13)	31.28 ± 1.85 (13)
Low Car- denolide	0.21	0.38 ± 0.04 (12)	1.17 ± 0.13 (12)	32.74 ± 2.26 (12)
Medium Car- denolide	0.53	0.39 ± 0.06 (14)	1.26 ± 0.16 (14)	30.98 ± 5.95 (14)
High Car- denolide	0.97	0.37 ± 0.08 (14)	1.23 ± 0.15 (14)	30.30 ± 5.91 (14)
Kruskal-Wallis χ^2		0.47	3.87	6.63
df		4	4	4
P		0.98	0.42	0.16

Table 14. Additional data for velvetbean caterpillars reared on artificial diets incorporating varying amounts of cardenolide. (A) Duration of the larval period from hatching until the start of the sixth instar. These observations terminated after 17 days. All larvae in the Control and Aerosol groups had reached the VI instar but 1, 2, and 5 larvae in the Low-, Medium-, and High-Cardenolide groups, respectively, had not. The medians given for these three groups omit these individuals and are therefore underestimated. (B) Estimated dry weights of newly-molted, fifth instars. The estimation is arrived at by multiplying the fresh weight by the dry weight:fresh weight ratio determined on a similarly reared sample of larvae. Means followed by the same letter are not significantly different at the 0.05 level by Duncan's multiple range test. (C) 2X5 contingency table showing the numbers of larvae dead or alive after 17 days feeding. Nine of these larvae died in the I or II instar, one in the III, and one in the V.

	TREATMENT				
	CARDENOLIDE				
	CONTROL	AEROSOL	LOW (0.21%)	MEDIUM (0.53%)	HIGH (0.97%)
(A) Larval Period (Hatching-VI)					
Median Days	13	13	14	13	14
Range	13-16	13-16	13-17+	12-17+	13-17+
N	22	22	25	23	21
(B) Estimated Dry Weight (V)					
$\bar{X}$	4.97 ^a	4.98 ^a	4.08 ^{ab}	3.71 ^b	3.76 ^b
S.D.	1.39	1.47	1.08	1.18	1.55
N	13	13	12	14	14
One-way ANOVA $F_{4,65}=2.94$, $P=0.03$					
(C) Survival After 17 Days					
No. Alive	29	30	29	26	25
No. Dead	1	0	1	4	5
$\chi^2 = 9.2$, $df = 4$, $P = 0.05$					

[Hemiptera: Lygaeidae]: Isman, 1977; Vaughan, 1979; Chaplin and Chaplin, 1981). In all but two cases (Vaughan, 1979; Seiber et al., 1980), the authors compared metabolic performance of insects when fed different species of milkweeds, that differed in mean cardenolide concentration. The problems associated with this approach have been elaborated elsewhere (see Chapter IV) and relate to a confounding of cardenolide and other nutritionally-important plant species differences. Other workers have studied cardenolide-effects upon lepidopteran neural tissue in vitro (Vaughan and Jungreis, 1977) or have utilized injected (rather than ingested) cardenolide (von Euw et al., 1967; Rafaeli-Bernstein and Mordue, 1977). While all three of these latter studies concluded that milkweed-feeding insects are less sensitive to cardenolide than are species that do not normally feed on milkweeds, their results must be regarded with caution because in vitro and injection assays circumvent much of the natural biology of the study organisms (see Chapter I). It is only when ingested by an intact animal that cardenolides can be expected to have their natural effects upon herbivores.

The data presented here demonstrate, for the first time, that ingested cardenolide can have negative effects upon non-adapted herbivores. Thus, the potential exists for cardenolides to constitute a chemical barrier against herbivory by at least some insect species. As predicted, the highly polyphagous fall armyworms grew more slowly as dietary cardenolide concentration increased (Table 11). This effect is attributable to two causes: (1) the larvae consumed less of

the medium- and high-cardenolide diets than of the control and low-cardenolide diets, and growth is obviously dependent, in part, upon consumption. A similar behavioral feeding inhibition was reported (albeit non-quantitatively) by Nielsen (1978) for chrysomelid beetles fed cardenolide-treated leaf discs. However, a behavioral inhibition against ingesting a particular diet (in the absence of alternative food sources) would only be adaptive if the diet is indeed detrimental (an issue not addressed by Nielsen, 1978). That this is the case in fall armyworms is reflected by the (2) decreased conversion efficiency of larvae fed the cardenolide-treated diets. Such larvae must spend additional time and energy in feeding behavior, relative to control larvae, in order to support the same amount of weight gain.

A second indication of a metabolic cost to fall armyworms of ingesting cardenolide is seen in the prolongation of the median larval period by as much as 20% (Table 12A). Indeed, some cardenolide-fed individuals required fully twice as long as typical control larvae to complete this developmental period. A slower development not only would expose the larvae for longer periods of time to predators, parasites, and other mortality agents, but can be related directly to a decrease in fitness through a reduction in the intrinsic rate of natural increase: $r = (\log_e R_0) / T$, in which R_0 is the net reproductive rate and T is the mean generation (or development) time. Thus, individuals having longer developmental periods will have lowered mean fitness unless this is accompanied by a very substantial increase in net reproductive rate (Green and Painter, 1975; Lanciani and May,

1982). However, since body weight and fecundity are well correlated in Lepidoptera (see Hinton, 1981), the tendency of armyworms fed high-cardenolide diets to weigh less than control animals suggests that such a compensatory increase in reproductive rate is highly unlikely.

If it is agreed that the most drastic effect an allelochemic can have on an herbivore is to cause direct mortality, then the legume-specializing velvetbean caterpillars, as predicted, were more severely affected than the polyphagous fall armyworms (compare Tables 12C and 14C). Moreover, while armyworms fed 1.2% cardenolide diets were able, by prolonging development, to maintain their body weights within 83% of control levels (Table 12B), velvetbean caterpillars fed only 0.53% cardenolide diets suffered a significant 25% mean decline in fifth instar body weight (Table 14B), despite a comparable increase in development time (Table 14A).

With these indications of a severe allelochemic impact on the negative-specialist, it is perhaps surprising that dietary cardenolide had no apparent effect upon consumption rate, growth rate, or conversion efficiency in the penultimate instar (Table 13). Clearly, then, the greatest effect is on the younger instars in this species. This difference in age-specific susceptibility may reflect the ontogenetic development of allelochemic-detoxification abilities (see Brattsten et al., 1977). While younger instars may succumb to cardenolide effects (Table 14C), those which survive to the penultimate stage (on which the gravimetric study was performed) may have had sufficient time to induce the detoxification enzymes

necessary to process cardenolides (see also Ahmad and Forgash, 1975). The truly surprising result may then be not that velvetbean caterpillars are unharmed by cardenolide in the penultimate instar but, rather, that fall armyworms are harmed, since polyphagous species appear to have more active detoxification systems than do oligophagous species (Krieger et al., 1971).

The strong effects of cardenolides upon younger velvetbean caterpillar instars, and their apparent absence in the penultimate stage serve as an important warning to other workers utilizing the gravimetric protocol (Waldbauer, 1968). Most studies of this nature investigate allelochemic-effects only upon the penultimate or ultimate instars (e.g., Erickson, 1973; Blau et al., 1978; Scriber and Feeny, 1979; Seiber et al., 1980; Futuyma and Wasserman, 1981). My data show that a failure to test for effects on younger instars can lead to very misleading results.

As also predicted, the milkweed-specializing monarch butterfly showed no apparent response to dietary cardenolide, even at concentrations 2.5 times greater than encountered in nature (Table 10). This confirms the results of Seiber et al. (1980) for both fourth and fifth instar monarchs. It should be noted that in both of these studies the larvae were reared on diets containing low to negligible cardenolide until the penultimate instar, when they were suddenly dosed with abnormally-high concentrations. This rearing design does not permit the testing of cardenolide-effects upon younger instars (see above). However, it minimizes the opportunity for the induction of detoxification enzymes by younger instars and thereby

maximizes the potential effect of the allelochemic during the gravimetric trials. Thus, the lack of an effect on monarch consumption rate, growth rate, or conversion efficiency reported here is even more dramatic.

The trend in monarchs toward higher consumption and growth rates with increasing dietary cardenolide concentration, while not statistically significant, may suggest an intermediate stage of adaptation to these allelochemicals. Many oligophagous insects utilize hostplant allelochemicals as feeding stimulants (e.g., Fraenkel, 1959; Rees, 1969; van Emden, 1972; Nielsen, 1978) and the possibility that monarchs are now in an early stage of evolving such a capacity warrants further research.

Cardenolide researchers have long speculated and argued over whether these allelochemicals evolved to defend plants against potential invertebrate or vertebrate herbivores. The data presented herein are the first to suggest that cardenolides may have evolved, at least in part, as a defense against certain insects. However, the existence of such a chemical barrier neither proves that the allelochemic originally evolved as a plant defense mechanism at all, nor that it is currently maintained because of its anti-insect effects. As correctly noted by Moran and Hamilton (1980) and Fox (1981), a defense (like most traits) will evolve only if it enhances the fitness of its possessor; it is not sufficient merely to diminish the fitness of one's enemies. Further work should next be directed at testing the effects of cardenolides upon plant fitness.

Finally, it must be noted that whereas the demonstration of a chemical barrier is necessary to coevolutionary models that assume their breaching (see Introduction), this is not evidence that such a breaching has necessarily occurred in the evolutionary history of specialist herbivores. An alternative is that extant milkweed specialists (or their progenitors) were biochemically preadapted for handling cardenolides and did not have to evolve this capacity during the course of evolutionary interaction with milkweed plants. This subject is discussed more fully in Chapter VI.

CHAPTER VI
GENERAL DISCUSSION AND CONCLUSION

General Discussion

The four chapters presented in this dissertation bear on the related questions of plant/herbivore coevolution and chemical defense in a system widely touted as a model for both. However, as described in earlier chapters, previous research has provided little direct evidence either for the coevolution of milkweed plants with their specialized herbivores, or for their chemical defense against generalist herbivores. It has been repeatedly stressed that a demonstration of a chemical barrier is a prerequisite for claims that specialist herbivores have breached such a defense during the course of coevolution with their hostplants. Clearly, this alone will not suffice to demonstrate coevolution (see below) but its absence (as has been the case with cardenolides until now) is sufficient to reject such a coevolutionary scenario.

I have used a variety of both observational and experimental techniques to investigate the effects of cardenolide upon the behavior and physiology of both milkweed-adapted and non-adapted lepidopteran species. Behavioral traits considered included female oviposition and

larval feeding rates. Physiological variables included larval survival, growth rate, food conversion efficiency, cardenolide sequestration, and relationships between adult cardenolide content and body size or weight. In Chapters II-IV, I focused upon adapted species since even these would be expected to show a range of tolerance for cardenolides reflecting different degrees of adaptation to these allelochemicals.

One method for assessing the physiological cost of ingesting and storing cardenolides is to search for correlations between the amount of the allelochemical sequestered and the adult body size or weight, since these latter variables appear to be correlated with fitness. Such a cost need not imply that the net benefit to cardenolide-ingesting insects is necessarily negative. Sequestered allelochemicals may defend the insect from predation or parasitism (see Chapters III and IV) and thereby increase fitness. However, a reduced body size may be considered an investment that the insect must make in order to achieve this level of defense. It is assumed that, as adaptation to allelochemicals evolves, the magnitude of the investment required would be reduced. In Chapter III, I reported strong negative correlations between the amount of cardenolide sequestered and both body size and weight of monarch butterflies, whether wild-caught or laboratory-reared. Since no such correlations were found for the congeneric queen butterfly, these data suggest that monarchs may pay physiological costs of cardenolide incorporation which queens do not. As discussed in Chapter III, this difference may be related to

differences in movement patterns and consequent degrees of inbreeding in the two species. Monarchs are highly migratory and congregate by the millions in Mexico prior to mating. They are therefore more likely than are queens to mate with individuals that have developed as larvae on different milkweed species. Since there is such great diversity in the amounts and kinds of cardenolides found in different milkweeds (many species lack these compounds entirely; see Chapter I), such interbreeding would tend to retard the rate of adaptation to cardenolides among monarchs. In contrast, queen butterflies make only regional movements within more localized geographic areas. This would likely result in a higher frequency of matings among individuals that had fed on the same larval foodplant species and would tend to promote a more rapid evolution of cardenolide-tolerance than would be expected among monarchs. This hypothesis receives further support from the case of the dogbane tiger moth (Chapter IV), another nonmigratory, milkweed-feeding species that showed no significant correlation between cardenolide content and body weight.

A second technique used to study the costs of feeding on cardenolide-rich plants involves the distribution of eggs in relation to the allelochemic content of the hostplants (Chapter II). This study was stimulated by the results of Dixon et al. (1978) who reported an apparent preference of monarch butterflies to oviposit on a lower-cardenolide milkweed species relative to higher-cardenolide species. Such a preference, if confirmed by more careful study, might suggest an aversion to plants of high allelochemic content, and lead

to subsidiary hypotheses to explain its evolution (e.g., a metabolic cost to the larvae). However, Dixon et al.'s experimental design confounded cardenolide differences with other milkweed species differences and the ovipositional preference could therefore not be attributed to the lower cardenolide content per se of the preferred milkweed species. In Chapter II, I approached this problem by studying egg distribution patterns in relation to intraspecific milkweed cardenolide variation, thereby removing a major source of confoundment (i.e., other plant species differences). This study failed to demonstrate any significant effect of plant cardenolide concentration upon egg deposition patterns. Moreover, estimates of larval success on these plants (which, if lowered on high-allelochemic plants, could reflect metabolic costs) were unrelated to the cardenolide content of the plants. The results on oviposition are in contrast to those of Dixon et al. (1978) and they offer no support for the hypothesis that monarchs pay metabolic costs of feeding on cardenolide-rich plants. However, the results are not necessarily incompatible with the earlier finding of a negative correlation between adult monarch cardenolide content and body size, since different life history stages were studied in each case. It is possible that cardenolides may not adversely affect larvae but may exert their effects during metamorphosis to the adult stage, thereby producing smaller adults. This possibility remains to be investigated.

A more direct, experimental test of cardenolide-effects upon behavior and physiology was reported in Chapter V in which one cardenolide (digitoxin) was added to the diets of three lepidopteran species: monarch butterflies, fall armyworms, and velvetbean caterpillars. It was predicted that fall armyworms, because they are highly polyphagous insects, should pay larger metabolic costs of cardenolide ingestion than should monarchs which, as a species, specialize on cardenolide-containing plants. Moreover, velvetbean caterpillars, which specialize on the Leguminosae (a plant family lacking cardenolides), should be most severely affected by cardenolides because, although they should have a finely-tuned detoxification ability, this would be directed at handling the allelochemicals of legumes rather than those of milkweeds. These predictions were generally supported by the results. Fourth instar monarchs showed no cardenolide-induced alterations in growth rate, feeding rate, or food conversion efficiency, even when fed diets containing cardenolide concentrations 2.5 times greater than those ever encountered in natural milkweeds. In contrast, fifth instar fall armyworms demonstrated reductions in growth, food consumption, conversion efficiency, and developmental rate, even at very moderate, natural cardenolide concentrations. While fifth instar velvetbean caterpillars were unaffected even by unusually high cardenolide concentrations, additional data revealed severe allelochemic effects upon the younger instars, including dose-dependent mortality.

Several aspects of this last study deserve further mention. Firstly, fourth instar monarchs did show a trend toward higher consumption (and hence, growth) rates as dietary cardenolide increased. While this trend was not significant, it could reflect an intermediate stage in the evolution of monarch adaptation to cardenolides. As noted in Chapter I, some oligophagous insects have been shown to utilize plant allelochemicals as attractants for either oviposition, larval feeding, or both. In the case of monarchs, cardenolides cannot be the critical feeding or oviposition stimulant, since these insects will recognize and use cardenolide-free milkweed species as food. However, cardenolides could nevertheless alter the insects' motivational level to feed or oviposit, once these behaviors have been released by the key stimuli. While my data provide no evidence that cardenolides influence oviposition behavior (Chapter II), the trend toward increased larval feeding rates shown in Chapter V is intriguing and merits further study.

Secondly, the effects of cardenolide on fall armyworms and velvetbean caterpillars were more pronounced at higher allelochemical concentrations than at lower levels. This dose-dependence serves as a warning regarding Feeny's (1975, 1976) dichotomy of plant chemical defenses as either qualitative or quantitative. According to Feeny's view, such compounds as tannins, which may occur in very high concentrations in plants (see Chapter I), are "quantitative" defenses in the sense that ever-higher concentrations may be selected for in plants in order to maintain a defense against herbivores which are

evolving, in parallel, ever-greater tannin-tolerance. In contrast, Feeny views such compounds as cardenolides as "qualitative" defenses. These typically occur in relatively low concentrations (<2% by dry weight) in plant tissues and may be toxic to a broad range of potential attackers. [They may be relatively easy barriers to overcome evolutionarily, however, as studies of pesticide-resistance suggest (see Futuyma, 1983).] Nevertheless, this need not mean that cardenolides are any less "quantitative" than tannins, and their now-demonstrated dose-dependent effects suggest that, like tanniniferous plants, milkweeds could be involved in quantitative evolutionary races against their enemies.

Are these races necessarily "coevolutionary"? As discussed in Chapter I, a strict definition of coevolution requires that at least three reciprocal evolutionary steps be identified. For example, a plant may evolve a chemical defense in response to herbivore pressure from an insect population (step 1). The insect may evolve a means of tolerating or circumventing the defense (step 2). The plant evolves yet another defense mechanism, or greater levels of the first mechanism (step 3). What is the evidence that the chemical defense of milkweeds has resulted from such a coevolutionary process? This question can be conveniently dissected into three subquestions, each corresponding to one of the above coevolutionary steps.

1) Against which species have milkweeds evolved cardenolides? This can only be inferred from identification of potential enemies

that are presently deterred or harmed by cardenolides. The most likely candidates for this are pathogenic organisms (e.g., bacteria or viruses), vertebrate herbivores, and invertebrate herbivores (notably insects). To date there is no evidence that cardenolide affects pathogenic organisms. Whereas there have been, to my knowledge, no studies with viruses as target organisms, Frings et al. (1948) reported that hemolymph of the milkweed bug (Oncopeltus fasciatus), extracted in saline, inhibited the in vitro growth of the bacteria Staphylococcus aureus and one strain of Bacillus subtilis. These results were later misinterpreted by Rothschild (1972) and by Price (1980) to be due to the presence of cardenolides in the hemolymph. However, a careful reading of the original study will show that saline extracts of the milkweed seeds (upon which the bugs were reared) had no antibacterial properties. Since the seeds are the source of cardenolides for milkweed bugs (e.g., Isman et al., 1977a), it is unlikely that the cardenolides were the antibacterial agent. Moreover, after solvent-partitioning of the hemolymph, Frings et al. (1948) found antibacterial action only in the water-soluble fraction and not in the lipophilic fraction. Since milkweeds commonly contain lipophilic cardenolides (e.g., Seiber et al., in press), this sheds further doubt that the hemolymph cardenolides were responsible for the antibacterial effects. In the only direct test to date of the possible antibacterial action of cardenolides, Malcolm (1981) grew the bacteria Escherichia coli and Bacillus cereus var. mycoides on culture media incorporating the cardenolides ouabain or digitoxin.

His results show no significant allelochemic effects upon colony growth for either bacterial species. Obviously, other pathogens might be affected by cardenolides but, if so, they have not yet been identified.

In contrast, there is a large body of pharmacological evidence that cardenolides are toxic to many vertebrate species (see Chapter I). However, most of this evidence is based upon intravenous injections in domesticated mammals (see Detweiler, 1967), rather than studies of the effects of ingested cardenolide on wild herbivores. Although there have been cases of poisoning of domestic livestock that have eaten milkweed plants (e.g., Tunnicliff and Cory, 1930; Campbell, 1931), such data for wild herbivores are lacking. Thus, it is not yet possible to speculate about which wild vertebrate species might have promoted the evolution of cardenolides in milkweeds. They may even have been representatives of now-extinct groups (see Janzen, 1980).

Finally, cardenolides might have evolved as a response to pressure from invertebrate herbivores. For example, one might hypothesize that interspecific differences in milkweed cardenolide concentration (Chapter I) reflect differential risks to milkweed-specializing insects. This would be suggested by the fact that the two north-Florida milkweed species (Asclepias humistrata and A. viridis) which have re-established widespread, above-ground populations by early spring (when large numbers of monarchs enter Florida from Mexico) have substantially higher cardenolide concentrations than do several other species which either grow more

sparsely or emerge later (after most of the migrants have left the area). If monarch herbivory has been a major cause of the high allelochemic levels in the two early species, we would expect monarchs to be either deterred or directly harmed by the levels of cardenolide now found in these plants. However, Chapter V shows that even considerably higher concentrations may have no adverse effect on monarchs. (Note that since only digitoxin-effects were tested, this result bears repetition with other cardenolides). Thus, at this stage, it is not possible to view present milkweed species cardenolide differences as resulting from differential risks of the plants to monarchs.

In contrast to monarchs, the fall armyworms and velvetbean caterpillars studied in Chapter V began to show adverse effects of cardenolides at levels between 0.17% and 0.56% (Chapter V). Interestingly, this range is approximately that found in the two early-spring milkweed species mentioned above (see Table 1). This suggests that non-adapted species, rather than specialists, may account for the present levels of cardenolide in milkweed plants. However, there is no evidence that any particular non-adapted species has interacted with any milkweed population on a persistent basis for sufficient time to have taken the next step in the coevolutionary sequence, i.e., the evolution of a specific counter-defense to cardenolides.

2) Have any milkweed enemies evolved specific counter-defenses to cardenolides? As implied above, this would be most likely to occur in oligophagous species, i.e., those that do not feed on other plant families and have no choice but to face the cardenolides. While my data suggest that specialists have greater cardenolide tolerance than generalists, it is by no means evident that they have evolved such tolerance during the course of their evolutionary interactions with milkweeds. One alternative is that specialists were biochemically preadapted for handling cardenolides. This hypothesis can be investigated via the comparative method. For example, certain genera of Danaidae (e.g., Amauris, Euploea) are reputed to feed primarily on cardenolide-free milkweed species (Rothschild and Marsh, 1978). If this is true, then such butterflies will not have recently undergone strong selection for cardenolide tolerance. Thus, if they are found to be harmed by cardenolides, it will imply that monarchs and other cardenolide-ingesting danaids have evolved cardenolide-tolerance during an evolutionary history of exposure to these allelochemicals. In contrast, if Amauris and Euploea, despite their feeding habits, are nevertheless found to have a cardenolide-tolerance similar to that of monarchs, it will imply either that (a) the entire family was preadapted for dealing with cardenolides and did not have to evolve this capacity, or (b) the tolerance evolved prior to the divergence of the genus Danaus from Amauris and Euploea and has not been lost despite relaxed selection for its maintenance. Possibility (b) (i.e., cardenolide-tolerance by descent from species that evolved such

tolerance) can be potentially eliminated by testing the tolerance of butterfly taxa not closely related to danaids and having no known history of interaction with cardenolide-containing plants (e.g., Pieridae, Papilionidae, Lycaenidae; Ehrlich and Raven, 1964). To the extent that these are nevertheless tolerant of cardenolides, it would suggest a general biochemical preadaptation among butterflies for dealing with these allelochemicals. My data (Chapter V) show that such preadaptation, if it exists, is not so broad among Lepidoptera as to include certain noctuid moths. However, it might well extend to pierid butterflies: in preliminary experiments, Feeny (pers. comm.) has found a considerable tolerance of Pieris rapae larvae to strophanthin cardenolides, thus supporting the preadaptation hypothesis. Of course, cardenolide-ingesting species could still evolve improvements upon their preadaptations. If future work shows this to be the case, it will suggest that the second step in the coevolutionary sequence has been taken.

Although sufficient work has not yet been done to test fully the preadaptation hypothesis in Lepidoptera, a revealing parallel situation exists at a higher trophic level. Deer mice (Peromyscus maniculatus labecula) are known to feed upon cardenolide-containing monarch butterflies overwintering in Mexico (Brower et al., in prep.). As in the case of insects attacking milkweeds (or birds attacking monarchs; Fink and Brower, 1981), one might well ask whether these rodents have evolved this ability or were preadapted for it. In laboratory experiments, Marty (1983) has shown that a subspecies of

deer mouse found in California (P. m. gambelii) has a high tolerance for ingested cardenolide, which is poorly absorbed across the gut. Since P. m. gambelii does not feed on cardenolide-containing insects, these results suggest that Peromyscus maniculatus may be a species generally preadapted for ingesting cardenolides safely. (Indeed, even laboratory rats are strongly resistant to cardenolides. Detweiler (1967) reported that the LD-50 for intravenous ouabain in this species was 671 times greater than that for domestic cats.) Whether P. m. labecula has evolved further refinements of this preadaptation is an important area for future research.

3) Have milkweed plants evolved any specific counter-defenses against their specialist enemies? Presumed examples of such counter-defense in non-milkweed species (described more fully in Chapter I) include mechanical structures (e.g., the egg-mimicking structures or hooked trichomes of Passiflora plants; Gilbert, 1983), higher concentrations of existing chemical defenses (e.g., tannins; Feeny, 1976), the addition of new kinds of chemical defenses to the arsenal (e.g., the addition of angular furanocoumarins to linear furanocoumarins in Umbelliferae; Berenbaum, 1983), or displacement in space and time to avoid specialist herbivores altogether (Rhoades and Gates, 1976). While milkweeds may show certain of these features, there is no evidence that these evolved as counter-adaptations to their specialized enemies. For example, Asclepias tuberosa in north-central Florida sprouts late in the spring, after most monarchs have

left the area on their northward migration (see Chapter I). While this certainly makes the plants unavailable to these herbivores, it is not known whether herbivore pressure is in any way responsible for this phenological pattern rather than, say, competition for pollinators (e.g., Pleasants, 1980). Ever-higher concentrations of cardenolides would seem to be effective against generalist insects but not as a counter-strategy against specialists (Chapter V). The addition of new cardenolides (e.g., of lower polarity; see Chapter I) might present a greater challenge to specialists but, again, there is no evidence that milkweed species or populations that are under greater specialist pressure have a predictably different set of cardenolides from those under less pressure. This is a major line of research that should be conducted.

The above considerations provide scant support for the contention that milkweeds have coevolved with their specialized herbivores in the kind of three-step manner described earlier. However, Edgar et al. (1974) have proposed an alternative coevolutionary scenario in which the progenitors of extant milkweed plants may have interacted with their specialist herbivores. They hypothesize that such plants contained (presumably, but not necessarily, for defensive purposes) both cardenolides and pyrrolizidine alkaloids (PA's) and that females of species comprising the basal stock of Danaidae and Ithomiidae utilized these chemicals as host recognition cues. Whereas the cardenolides (and possibly the PA's as well) provided the insects with a potent chemical defense, the PA's became further necessary as

precursors of male sex pheromones (see references in Chapter III), possibly because this allowed males to induce females to land away from their hostplants (and therefore to be mated). Once the butterflies had become dependent upon both PA's and cardenolides for these purposes, those plants which lacked either one or both of these chemical classes would be favored by natural selection because they would attract fewer herbivores. As a result, this group of plants gave rise phylogenetically to three branches, one containing cardenolides only (Apocynales: Asclepiadaceae and Apocynaceae), one containing PA's only (Boraginaceae and Compositae), and one containing neither (Solanaceae). [All five of these plant families are placed in a single subclass, Asteridae (Cronquist, 1968; Takhtajan, 1969), suggesting a common origin.] The divergence of the plants could then have been followed by a divergence of the butterfly stock into apocynale-specialists (danaids) and Solanaceae-specialists (ithomiids), with adults of both families still visiting PA-containing plants to acquire pheromonal precursors and/or defensive compounds. This hypothesis is coevolutionary in that the following three fundamental steps are included. (1) The primitive butterflies evolved pheromonal systems based upon chemicals already present in their larval foodplants. [It is not necessary that these chemicals evolved as defenses against these particular butterflies.] (2) The herbivore pressure led to a deletion of either PA's or cardenolides (or both) from the plants. Since selection would presumably act against hybrid plants containing both chemical classes, an evolutionary divergence

resulted. (3) The butterflies diverged in parallel to the plants, giving rise to the danaids and ithomiids. These three steps, if documented, would satisfy even Janzen's (1980) rigorous criteria for coevolution (see Chapter I).

The strongest support for Edgar et al.'s (1974) ingenuous hypothesis comes from the observation that certain primitive, danaid-like, extant ithomiids have been discovered to feed as larvae on PA-containing plants in the tribe Parsonieae of the Apocynaceae. Thus, neotropical ithomiids in the genera Tithorea and Aeria feed upon plants in the genera Fernaldia and Prestonia (Edgar, 1982). Both plant genera include species containing PA's and some Fernaldia species are known to contain cardenolides (Edgar et al., 1974). It therefore seems plausible that at least certain plants fed upon by primitive-stock butterflies contained cardenolides and PA's simultaneously, as required by the hypothesis. Moreover, whereas in most danaid and ithomid butterflies it is predominantly adult males which are attracted to withering PA-sources, in Tithorea females are equally attracted (Pliske, 1975). This suggests that while females of other genera have lost their presumably primitive responsivity to PA's (because these no longer occur in their solanaceous hostplants), females of species still feeding as larvae on PA-containing plants have not. However, it has not yet been established whether Tithorea females, as predicted, utilize PA's as hostplant recognition cues.

A further observation adding credence to the coevolutionary hypothesis is of another primitive danaid-like ithomid, this one from

Australia and New Guinea. Larvae of this species, Tellervo zoilus zoilus, feed upon the PA-containing apocynad, Parsonsia velutina (also in the tribe Parsonieae). The fact that the Tellervo-Parsonsia association is found in Australia, which has been separated from the neotropics since the breakup of Gondwanaland in the Eocene, strongly suggests that these associations between primitive ithomiines and PA-containing plants in the Parsonieae, both in the new and old world, are representative of an ancestral condition. Whereas Edgar et al. (1974) and Edgar (1982) do not present evidence that the tribe Parsonieae, like its ithomiine herbivores, is primitive, the placement of all five of the plant families concerned in the hypothesis into the single subclass, Asteridae, implies the kind of phylogenetic radiation hypothesized by these authors. Moreover, certain PA's found in Parsonsia are typical of the Boraginaceae and of the genus Eupatorium of the Compositae (Edgar and Culvenor, 1975). These facts lend yet further support to Edgar et al.'s (1974) hypothesis.

The above hypothesis has been challenged by Boppré (1978), who offered a non-coevolutionary alternative interpretation of the data. He suggests that primitive-stock danaid/ithomiid adults (of both sexes) fed occasionally on PA-containing nectar. Those individuals with a tendency to accumulate PA's in external tissues (which would permit them to be tasted by predators and released unharmed; see Brower, in press) would be favored by selection. Eventually, wilting leaves of certain plants were discovered by the butterflies to be more reliable PA-sources. Boppré (1978) further suggests that females may

have evolved an attraction to males disseminating "PA-odors" as a means of avoiding courtship and possible hybridization with males of mimicking species. This could have led to the incorporation of PA's into the pheromonal system. Finally, he proposes that the use by danaids of cardenolide-containing plants was an independent evolutionary development.

As evidence for his hypothesis, Boppre (1978) points out that PA's have been found in the nectar of certain Senecio plants (Compositae) (Deinzer et al., 1977) fed upon by adult danaids. He further notes that the (occasional) use by danaids of Crotalaria (Leguminosae) and some Moraceae as adult PA-sources and larval foodplants, respectively, does not fit with Edgar et al.'s (1974) hypothesis and suggests that the use of PA's in the pheromonal systems of these butterflies evolved independently of the phylogeny of larval foodplants.

It is important to note that Edgar et al.'s (1974) hypothesis does not assume that cardenolides (or PA's) evolved in the plants as deterrents to lepidopteran (or other) herbivores but, rather, that they were deleted from some plants because they made the plants more conspicuous to females searching out larval hostplants. Such a deletion would presumably have resulted from selection favoring those individual plants in a population that had somewhat lower cardenolide concentrations than others (assuming heritability of plant cardenolide content). If female butterflies utilized cardenolides for host-finding, then such lower-cardenolide plants would presumably have been located and oviposited upon less frequently than higher-cardenolide

plants. This situation was effectively modelled in Chapter II in which it was found that oviposition rates of monarch butterflies are independent of plant cardenolide concentration. These results therefore do not support Edgar et al.'s (1974) hypothesis.

Boppré's (1978) alternative, however, cannot adequately explain the occurrence, in both Australia and the neotropics, of primitive ithomiines that utilize PA- (and probably also cardenolide-) containing larval hostplants. Edgar et al.'s (1974) hypothesis, having the benefit of phylogenetic and biogeographic data, is more persuasive in this respect and provides a reasonable, testable scenario in which the primitive hostplants of danaid and ithomiid butterflies may have coevolved (sensu stricto) with their specialist herbivores.

Conclusion

In conclusion, there is little evidence that the cardenolides of milkweed plants have evolved as deterrents to specialist herbivores, whether through a stepwise coevolutionary process or otherwise. However, my data show that these allelochemicals are deterrent and toxic to at least one generalist lepidopteran species and to another lepidopteran specialized for feeding on a plant family lacking cardenolides (Chapter V). Furthermore, the observed concentrations of cardenolides in the leaves of many milkweed species (Chapter I) are sufficient to cause negative metabolic effects upon such non-adapted

species but are apparently insufficient to harm adapted specialists. In the case of one non-adapted species (velvetbean caterpillar) cardenolide-effects were seen only in the younger instars but not in the penultimate stage. This serves as an important warning to other researchers utilizing the gravimetric protocol (Waldbauer, 1968) since most studies of this nature investigate allelochemic-effects only in the penultimate or ultimate instars (e.g., Erickson, 1973; Blau et al., 1978; Scriber and Feeny, 1979; Seiber et al., 1980; Futuyma and Wasserman, 1981). My data show that failure to test for effects on younger instars can lead to very misleading results. Furthermore, the dose-dependence of the responses to cardenolide in the two non-adapted species caution against a strict dichotomy of qualitative vs. quantitative allelochemicals (Feeny, 1975, 1976).

It is important in coevolutionary studies to specify the particular coevolutionary model being tested. In this dissertation I have considered two such models that have been proposed to account for the relationships between milkweeds and their specialist herbivores. The first of these might be called the "barrier-breach" model, i.e., the plants evolved cardenolides as a chemical barrier to insect herbivory. It is proposed that this barrier was later breached by the progenitors of extant milkweed specialists, which then led to the evolution of further plant defenses, etc. I have presented the first evidence that such a cardenolide barrier exists and may deter herbivory by certain non-adapted insects. It is, however, not known whether the cardenolide-tolerance exhibited by specialists evolved

during the course of evolutionary interaction with their hostplants (suggesting "barrier-breach") or reflects, instead, a biochemical preadaptation for coping with cardenolides. Recent evidence from other workers lends credence to the preadaptation hypothesis. Thus, claims of "barrier-breach" coevolution among milkweeds and their specialized herbivores are not supported by the available evidence.

A second model of coevolution among these organisms (Edgar et al., 1974) carries the weight of both phylogenetic and biogeographic evidence. This is a "co-speciation" model which does not require that cardenolides evolved in plants as insect-deterrents. Rather, it is hypothesized that herbivores utilized cardenolides and PA's for hostplant recognition and that this led to a divergence of the basal plant stock into three branches (details above). This, in turn, was followed by a divergence of the herbivore stock to specialize on the new plant diversity. One prediction of this "cospeciation model" is that cardenolides are at least partly responsible for hostplant attractiveness to specialist herbivores. Whereas my data on monarch oviposition rates in relation to plant cardenolides (Chapter II) argue against this, similar tests should be undertaken with other danaid (and ithomiid) species. Of particular interest are the primitive species that utilize the apocynaceous tribe Parsonieae as larval foodplants.

Finally, although milkweed plants and their herbivores (notably monarch butterflies) comprise one of the most thoroughly studied plant/herbivore associations, providing many insights into chemical

ecology, we are only now beginning to explore the details of these interactions. Because the chemistry of cardenolides is so well known (thanks to their medicinal properties), and techniques for their study are straightforward, one can expect that further work on the milkweed system will indeed be valuable in providing a clearer understanding of the evolution of plant/herbivore interactions.

LITERATURE CITED

- Ahmad, S., and Forgash, A.J. 1975. Toxicity of carbaryl and diazinon to gypsy moth larvae: changes in relation to larval growth. J. Econ. Ent. 68:803-806.
- Auerbach, M.J., and Strong, D.R. 1981. Nutritional ecology of Heliconia herbivores: experiments with plant fertilization and alternative hosts. Ecol. Monogr. 51:63-83.
- Beall, G. 1948. The fat content of a butterfly, Danaus plexippus Linn., as affected by migration. Ecology 29:80-94.
- Berenbaum, M.R. 1981. Patterns of furanocoumarin production and insect herbivory in a population of wild parsnip (Pastinaca sativa L.) Oecologia 49:236-244.
- Berenbaum, M.R. 1983. Coumarins and caterpillars: a case for coevolution. Evolution 37:163-179.
- Bernays, E.A. 1981. Plant tannins and herbivores: a reappraisal. Ecol. Entomol. 6:353-360.
- Bernays, E.A., Chamberlain, D.J., and McCarthy, P. 1980. The differential effects of ingested tannic acid on different species of Acridoidea. Ent. exp. appl. 28:158-166.
- Bisset, G.W., Frazer, J.F.D., Rothschild, M., and Schachter, M. 1960. A pharmacologically active choline ester and other substances in the garden tiger moth, Arctia caja (L.). Proc. R. Soc. (B) 152:255-262.
- Blau, P.A., Feeny, P., Contardo, L., and Robson, D.S. 1978. Allylglucosinolate and herbivorous caterpillars: a contrast in toxicity and tolerance. Science 200:1296-1298.
- Blum, M.S. 1981. Chemical defenses of arthropods. Academic Press, New York.
- Boppré, M. 1978. Chemical communication, plant relationships, and mimicry in the evolution of danaid butterflies. Ent. exp. appl. 24:264-277.
- Brattsten, L.B., Wilkinson, C.F., and Eisner, T. 1977. Herbivore-plant interactions: mixed function oxidases and secondary plant substances. Science 196:1349-1352.

- Brower, J.V.Z. 1958a. Experimental studies of mimicry in some North American butterflies. I. The monarch Danaus plexippus and viceroy Limnitis archippus. *Evolution* 11:32-47.
- Brower, J.V.Z. 1958b. Experimental studies of mimicry in some North American butterflies. III. Danaus gilippus berenice and Limnitis archippus floridensis. *Evolution* 12:273-285.
- Brower, L.P. 1961. Studies on the migration of the monarch butterfly. I. Breeding populations of Danaus plexippus and D. gilippus berenice in south central Florida. *Ecology* 42:76-83.
- Brower, L.P. 1969. Ecological chemistry. *Sci. Amer.* 220:22-29.
- Brower, L.P. In press. Chemical defence in butterflies. *Symp. R. Entomol. Soc.*
- Brower, L.P., and Brower, J.V.Z. 1964. Birds, butterflies, and plant poisons: a study in ecological chemistry. *Zoologica* 49:137-159.
- Brower, L.P., Brower, J.V.Z., and Corvino, J.M. 1967. Plant poisons in a terrestrial food chain. *Proc. Nat. Acad. Sci. USA* 57:893-898.
- Brower, L.P., M. Edmunds, and Moffitt, C.M. 1975. Cardenolide content and palatability of a population of Danaus chrysippus butterflies from West Africa. *J. Entomol. (A)* 49:183-196.
- Brower, L.P., Gibson, D.O., Moffitt, C.M., and Panchen, A.L. 1978. Cardenolide content of Danaus chrysippus butterflies from three regions of East Africa. *Biol. J. Linn. Soc.* 10:251-273.
- Brower, L.P., and Glazier, S.C. 1975. Localization of heart poisons in the monarch butterfly. *Science* 188:19-25.
- Brower, L.P., and Huberth, J.C. 1977. Strategy for survival: behavioral ecology of the monarch butterfly. 16 mm film. Harper and Row Media Inc., New York.
- Brower, L.P., McEvoy, P.B., Williamson, K.L., and Flannery, M.A. 1972. Variation in cardiac glycoside content of monarch butterflies from natural populations in Eastern North America. *Science* 177:426-429.
- Brower, L.P., and Moffitt, C.M. 1974. Palatability dynamics of cardenolides in the monarch butterfly. *Nature* 249:280-283.
- Brower, L.P., Ryerson, W.N., Coppinger, L.L., and Glazier, S.C. 1968. Ecological chemistry and the palatability spectrum. *Science* 161:1349-1351.

- Brower, L.P., Seiber, J.N., Nelson, C.J., Lynch, S.P., Hoggard, M.P., and Cohen, J.A. In prep. Plant-determined variation in cardenolide content and thin layer chromatography profiles of monarch butterflies, Danaus plexippus, reared on the milkweed, Asclepias californica in California.
- Brower, L.P., Seiber, J.N., Nelson, C.J., Lynch, S.P., and Tuskes, P.M. 1982. Plant determined variation in the cardenolide content, thin layer chromatography profiles, and emetic potency of monarch butterflies, Danaus plexippus, reared on the milkweed, Asclepias eriocarpa in California. J. Chem. Ecol. 8:579-633.
- Brower, L.P., Seiber, J.N., Nelson, C.J., Lynch, S.P., and Holland, M. In press. Plant determined variation in cardenolide content, thin layer chromatography profiles, and emetic potency of monarch butterflies, Danaus plexippus, reared on the milkweed, Asclepias speciosa in California. J. Chem. Ecol.
- Burns, J.M. 1983. Queen of the Carolinas (Lepidoptera: Nymphalidae: Danainae: Danaus gilippus). Proc. Entomol. Soc. Wash. 85:388-396.
- Campbell, H.W. 1931. Poisoning in chickens with whorled milkweed. J. Am. Vet. Med. Assn. 79:102-104.
- Chaplin, S.J., and Chaplin, S.B. 1981. Growth dynamics of a specialized milkweed seed feeder (Oncopeltus fasciatus) on seeds of familiar and unfamiliar milkweeds (Asclepias spp.). Ent. exp. appl. 29:345-356.
- Chew, F.S., and Rodman, J.E. 1979. Plant resources for chemical defense. In: G.A. Rosenthal and D.H. Janzen (eds) 1979, Herbivores: their interaction with secondary plant metabolites. Academic Press, New York.
- Cohen, J.A., and Brower, L.P. 1982. Oviposition and larval success of wild monarch butterflies (Lepidoptera: Danaidae) in relation to hostplant size and cardenolide content. J. Kans. Entomol. Soc. 55:343-348.
- Cohen, J.A., and Brower, L.P. 1983. Cardenolide sequestration by the dogbane tiger moth (Cycnia tenera; Arctiidae). J. Chem. Ecol. 9:521-532.
- Cronquist, A. 1968. The evolution and classification of flowering plants. Nelson, London.
- Daloze, D., and Pasteels, J.M. 1979. Production of cardiac glycosides by chrysomelid beetles and larvae. J. Chem. Ecol. 5:63-77.

- Dawkins, R., and Krebs, J.R. 1979. Arms races between and within species. *Proc. R. Soc. Lond. (B)* 205:489-511.
- Deinzer, M.L., Thompson, P.A., Burgett, D.M., and Isaacson, D.L. 1977. Pyrrolizidine alkaloids: their occurrence in honey from tansy ragwort (Senecio jacobae L.). *Science* 195:497-499.
- Detweiler, D.K. 1967. Comparative pharmacology of cardiac glycosides. *Fed. Proceed.* 26:1119-1124.
- Dixon, C.A., Erickson, J.M., Kellett, D.N., and Rothschild, M. 1978. Some adaptations between Danaus plexippus and its food plant, with notes on Danaus chrysippus and Euploea core (Insecta:Lepidoptera). *J. Zool., Lond.* 185:437-467.
- Duffey, S.S. 1977. Arthropod allomones: chemical effronteries and antagonists. XV Int. Congr. Entomol. 1976. Washington, D.C.
- Duffey, S.S., and Scudder, G.G.E. 1972. Cardiac glycosides in North American Asclepiadaceae, a basis for unpalatability in brightly coloured Hemiptera and Coleoptera. *J. Insect Physiol.* 18:63-73.
- Dunning, D.C., and Roeder, K.D. 1965. Moth sounds and the insect-catching behavior of bats. *Science* 147:173-174.
- Edgar, J.A. 1975. Danainae (Lep.) and 1,2-dehydropyrrolizidine alkaloid-containing plants -- with reference to observations made in the New Hebrides. *Phil. Trans. R. Soc. (B)* 272:467-476.
- Edgar, J.A. 1982. Pyrrolizidine alkaloids sequestered by Solomon Island Danaine butterflies. The feeding preferences of the Danainae and Ithomiinae. *J. Zool.* 196:385-399.
- Edgar, J.A., Boppre, M. and Schneider, D. 1979. Pyrrolizidine alkaloid storage in African and Australian danaid butterflies. *Experientia* 35:1447-1448.
- Edgar, J.A., Cockrum, P.A. and Frahn, J.L. 1976. Pyrrolizidine alkaloids in Danaus plexippus L. and Danaus chrysippus L. *Experientia* 32:1535-1537.
- Edgar, J.A., and Culvenor, C.C.J. 1975. Pyrrolizidine alkaloids in Parsonsia species (family Apocynaceae) which attract danaid butterflies. *Experientia* 31:393-394.
- Edgar, J.A., Culvenor, C.C.J., and Pliske, T.E. 1974. Coevolution of Danaid butterflies with their host plants. *Nature* 250:646-648.
- Edmunds, M. 1974. Defence in animals. Longman, Harlow (UK).

- Ehrlich, P.R., and Raven, P.H. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18:586-608.
- Erickson, J.M. 1973. The utilization of various Asclepias species by larvae of the monarch butterfly, Danaus plexippus. *Psyche* 80:230-244.
- Feeny, P.P. 1970. Seasonal changes in oakleaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* 51:656-681.
- Feeny, P.P. 1975. Biochemical coevolution between plants and their insect herbivores. In: L.E. Gilbert and P.H. Raven (eds), *Coevolution of animals and plants*. Univ. of Texas Press, Austin.
- Feeny, P.P. 1976. Plant apparency and chemical defense. *Rec. Adv. Phytochem.* 10:1-40.
- Fink, L.S. and Brower, L.P. 1981. Birds can overcome the cardenolide defence of monarch butterflies in Mexico. *Nature* 291:67-70.
- Forbes, W.T.M. 1960. Lepidoptera of New York and neighboring states, Part IV. Cornell Univ. Agric. Exp. Sta., N.Y. State Coll. Agric., Ithaca, N.Y.
- Fox, L.R. 1981. Defense and dynamics in plant-herbivore systems. *Am. Zool.* 24:853-864.
- Fox, L.R., and Macauley, B.J. 1977. Insect grazing on Eucalyptus in response to variation in leaf tannins and nitrogen. *Oecologia* 29:145-162.
- Fox, L.R., and Morrow, P.A. 1981. Specialization: species property or local phenomenon? *Science* 211:887-893.
- Fraenkel, G.S. 1959. The raison d'être of secondary plant substances. *Science* 129:1466-1470.
- Fraenkel, G.S. 1969. Evaluations of our thoughts on secondary plant substances. *Ent. exp. appl.* 12:473-486.
- Frazer, J.F.D. 1965. The cause of urtication produced by larval hairs of Arctia caja (L.) (Lepidoptera: Arctiidae). *Proc. R. Ent. Soc. Lond. (A)* 40:96-100.
- Frings, H., Goldberg, E., and Arentzen, J.C. 1948. Antibacterial action of the blood of the large milkweed bug. *Science* 108:689-690.
- Fullard, J.H. 1977. Phenology of sound-producing arctiid moths and the activity of insectivorous bats. *Nature* 267:42-43.

- Futuyma, D.J. 1979. Evolutionary biology. Sinauer Assoc., Sunderland, Ma.
- Futuyma, D.J. 1983. Evolutionary interactions among herbivorous insects and plants. In: D.J. Futuyma and M. Slatkin (eds), Coevolution. Sinauer Assoc., Sunderland, Ma.
- Futuyma, D.J., and M. Slatkin, 1983. Introduction. In: D.J. Futuyma and M. Slatkin (eds), Coevolution. Sinauer Assoc., Sunderland, Ma.
- Futuyma, D.J., and Wasserman, S.S. 1981. Food plant specialization and feeding efficiency in the tent caterpillars Malacosoma disstria and M. americanum. Ent. exp. appl. 30:106-110.
- Gilbert, L.E. 1975. Ecological consequences of a coevolved mutualism between butterflies and plants. In: L.E. Gilbert and P.H. Raven (eds), Coevolution of animals and plants. Univ. of Texas Press, Austin.
- Gilbert, L.E. 1979. Development of theory in the analysis on insect-plant interactions. In: D.J. Horn, R.D. Mitchell, and G.R. Stairs (eds.). Analysis of ecological systems. Ohio State Univ. Press, Columbus.
- Gilbert, L.E. 1983. Coevolution and mimicry. In: D.J. Futuyma and M. Slatkin (eds), Coevolution. Sinauer Assoc., Sunderland, Ma.
- Green, R., and Painter, P.R. 1975. Selection for fertility and development time. Am. Nat. 109:1-10.
- Hamilton, W.D. 1964. The genetical evolution of social behaviour. I, II. J. theor. Biol. 7:1-52.
- Harborne, J.B. 1983. Introduction to ecological biochemistry. 2nd edit., Academic Press, New York.
- Harvey, P.H., Bull, J.J., Pemberton, M., and Paxton, R.J. 1982. The evolution of aposematic coloration in distasteful prey: a family model. Am. Nat. 119: 710-719.
- Hatchett, J.H., and Gallun, R.L. 1970. Genetics of the ability of the Hessian fly, Mayetiola destructor to survive on wheats having different genes for resistance. Ann. Ent. Soc. Am. 63:1400-1407.
- Helwig, J.T., and Council, K.A. 1979. SAS user's guide. SAS Institute Inc., Cary, N.C. (USA).
- Hinton, H.E. 1981. Biology of insect eggs. Vol. 1. Pergamon Press, Oxford.

- Hodges, R.W. In press. A checklist of moths of North America. E.W. Classey, London.
- Hoffman, B.F., and Bigger, J.T. 1980. Digitalis and allied cardiac glycosides. In: A.G. Gilman, L.S. Goodman, and A. Gilman (eds), The pharmacological basis of therapeutics. Macmillan, New York.
- Isman, M.B. 1977. Dietary influence of cardenolides on larval growth and development of the milkweed bug Oncopeltus fasciatus. J. Insect Physiol. 23:1183-1187.
- Isman, M.B., Duffey, S.S., and Scudder, G.G.E. 1977a. Variation in the cardenolide content of the lygaeid bugs, Oncopeltus fasciatus and Lygaeus kalmii kalmii and of their milkweed hosts (Asclepias spp.) in central California. J. Chem. Ecol. 3:613-624.
- Isman, M.B., Duffey, S.S., and Scudder, G.G.E. 1977b. Cardenolide content of some leaf- and stem-feeding insects on temperate North American milkweeds (Asclepias spp.). Can. J. Zool. 55:1024-1028.
- Janzen, D.H. 1980. When is it coevolution? Evolution 34:611-612.
- Jermy, T. 1976. Insect-host-plant relationship- co-evolution or sequential evolution? Symp. Biol. Hung. 16:109-113.
- Kimball, C.P. 1965. The Lepidoptera of Florida. Div. Plant Industry, State of Fla. Dept. Agric., Gainesville, Fla.
- Kingsbury, J.M. 1964. Poisonous plants of the United States and Canada. Prentice-Hall, New York.
- Klots, A.B. 1951. A field guide to the butterflies. Houghton-Mifflin, New York.
- Krebs, J.R. 1978. Optimal foraging: decision rules for predators. In: J.R. Krebs and N.B. Davies (eds), Behavioural ecology: an evolutionary approach. Sinauer Assoc., Sunderland, Ma.
- Krieger, R.I., Feeny, P.P., and Wilkinson, C.F. 1971. Detoxification enzymes in the guts of caterpillars: an evolutionary answer to plant defenses? Science 172:579-581.
- Lanciani, C.A., and May, P.G. 1982. Parasite-mediated reductions in the growth of nymphal backswimmers. Parasitology 85:1-7.
- Langenheim, J.H., Foster, C.E., and McGinley, R.B. 1980. Inhibitory effects of different quantitative compositions of Hymenaea leaf resins on a generalist herbivore, Spodoptera exigua. Biochem. Syst. Ecol. 8:385-396.

- Leppla, N.C., Vail, P.V., and Rye, J.R. In press. Mass rearing the cabbage looper. In: E.G. King and N.C. Leppla (eds), *Advances and challenges in insect rearing*. USDA Tech. Bull., Washington, D.C.
- Levin, D.A. 1976. The chemical defenses of plants to pathogens and herbivores. *Ann. Rev. Ecol. Syst.* 7:121-159.
- Levins, R. 1968. *Evolution in changing environments*. Princeton Univ. Press, Princeton.
- MacArthur, R.H. 1972. *Geographical ecology: patterns in the distribution of species*. Harper and Row, New York.
- Malcolm, S.B. 1981. Defensive use of plant-derived cardenolides by Aphis nerii Boyer de Fonscolombe against predation. D. Phil. dissertation, Oxford Univ., Oxford.
- Marty, M.A. 1983. Metabolism and disposition of milkweed (Asclepiadaceae) cardenolides in monarch butterfly larvae, Danaus plexippus, and in a predator, Peromyscus maniculatus. Ph.D. dissertation, Univ. of California, Davis..
- Meinwald, J., Meinwald, Y.C. and Mazzocchi, P.H. 1969. Sex pheromone of the queen butterfly: chemistry. *Science*, 164, 1174-1175.
- Moran, N., and Hamilton, W.D. 1980. Low nutritive quality as defense against herbivores. *J. theor. Biol.* 86:247-254.
- Nelson, C.J., Seiber, J.N., and Brower, L.P. 1981. Seasonal and intraplant variation of cardenolide content in the California milkweed, Asclepias eriocarpa, and implications for plant defense. *J. Chem. Ecol.* 7:981-1010.
- Nielsen, J.K. 1978. Host plant discrimination within Cruciferae: feeding responses of four leaf beetles (Coleoptera: Chrysomelidae) to glucosinolates, cucurbitacins and cardenolides. *Ent. exp. appl.* 24:41-54.
- Nishio, S. 1980. The fates and adaptive significance of cardenolides sequestered by larvae of Danaus plexippus (L.) and Cycnia inopinatus (Hy. Edwards). Ph.D. Dissertation, Univ. of Georgia, Athens.
- Parsons, J.A. 1965. A digitalis-like toxin in the monarch butterfly, Danaus plexippus L. *J. Physiol.* 178:290-304.

- Pasteels, J.M., and Daloze, D. 1977. Cardiac glycosides in defensive secretions of chrysomelid beetles: evidence for their production by the insects. *Science* 197:70-72.
- Peters, T.M., and Barbosa, P. 1977. Influence of population density on size, fecundity, and developmental rate of insects in culture. *Ann. Rev. Entomol.* 22:431-450.
- Pleasants, J.M. 1980. Competition for bumblebee pollinators in Rocky Mountain plant communities. *Ecology* 61:1446-1459.
- Pliske, T.E. 1975. Attraction of Lepidoptera to plants containing pyrrolizidine alkaloids. *Environ. Entomol.* 4:455-473.
- Pliske, T.E., and Eisner, T. 1969. Sex pheromone of the queen butterfly: biology. *Science* 164:1170-1172.
- Pough, F.H., Brower, L.P., Meck, H.R., and Kessell, S.R. 1973. Theoretical investigations of automimicry: multiple trial learning and the palatability spectrum. *Proc. Nat. Acad. Sci. USA* 70:2261-2265.
- Price, P.W. 1980. *Evolutionary biology of parasites.* Princeton Univ. Press, Princeton.
- Price, P.W., and Willson, M.F. 1979. Abundance of herbivores on six milkweed species in Illinois. *Am. Midl. Nat.* 101:76-86.
- Pyke, G.H., Pulliam, H.R., and Charnov, E.L. 1977. Optimal foraging: a selective review of theory and tests. *Q. Rev. Biol.* 52:137-154.
- Rafaelli-Bernstein, A., and Mordue, W. 1978. The transport of the cardiac glycoside ouabain by the Malpighian tubules of Zonocerus variegatus. *Physiol. Entomol.* 3:59-63.
- Rees, C.J.C. 1969. Chemoreceptor specificity associated with choice of feeding site by the beetle Chrysolina brunsvicensis on its food-plant Hypericum hirsutum. *Ent. exp. appl.* 12:565-583.
- Reichstein, T., von Euw, J., Parsons, J.A., and Rothschild, M. 1968. Heart poisons in the monarch butterfly. *Science* 161:861-866.
- Remington, C.L. 1968. Suture-zones of hybrid interaction between recently joined biotas. In: T. Dobzhansky, M.K. Hecht, and W.C. Steere (eds), *Evolutionary biology*. Vol. 2. Appleton-Century-Crofts, New York.

- Rhoades, D.F. 1979. Evolution of plant chemical defense against herbivores. In: G.A. Rosenthal and D.H. Janzen (eds), *Herbivores: their interaction with secondary plant metabolites*. Academic Press, New York.
- Rhoades, D.F., and R.G. Cates. 1976. A general theory of plant antiherbivore chemistry. *Rec. Adv. Phytochem.* 10:168-213.
- Roeske, C.N., Seiber, J.N., Brower, L.P., and Moffitt, C.M. 1976. Milkweed cardenolides and their comparative processing by monarch butterflies (Danaus plexippus L.). *Rec. Adv. Phytochem.* 10, 93-167.
- Rosenthal, G.A., and Janzen, D.H. 1979. *Herbivores: their interaction with secondary plant metabolites*. Academic Press, New York.
- Rothschild, M. 1972. Some observations on the relationship between plants, toxic insects and birds. In: J.B. Harborne (ed.), *Phytochemical ecology*. Academic Press, New York.
- Rothschild, M. 1973. Secondary plant substances and warning colouration in insects. In: H.F. van Emden (ed.), *Insect/plant relationships*. Symp. R. Entomol. Soc. Lond. (Vol. 6). Blackwell, Oxford.
- Rothschild, M., Alpin, R.T., Cockrum, P.A., Edgar, J.A., Fairweather, P., and Lees, R. 1979. Pyrrolizidine alkaloids in arctiid moths (Lep.) with a discussion on host plant relationships and the role of these secondary plant substances in the Arctiidae. *Biol. J. Linn. Soc.* 12:305-326.
- Rothschild, M., von Euw, J., Reichstein, T., Smith, D.A.S., and Pierre, J. 1975. Cardenolide storage in Danaus chrysippus (L.) with additional notes on D. plexippus (L.). *Proc. R. Soc. Lond. (B)* 190:1-31.
- Rothschild, M., and Kellett, D.N. 1972. Reactions of various predators to insects storing heart poisons (cardiac glycosides) in their tissues. *J. Entomol. (A)* 46:103-110.
- Rothschild, M., and Marsh, N. 1978. Some peculiar aspects of *Danaid/plant* relationships. *Ent. exp. appl.* 24:437-450.
- Rothschild, M., Marsh, N., and Gardiner, B. 1978. Cardioactive substances in the monarch butterfly and Euploea core reared on leaf-free artificial diet. *Nature* 275:649-650.

- Rothschild, M., and Reichstein, T. 1976. Some problems associated with the storage of cardiac glycosides by insects. *Nova Acta Leopold.* Suppl. 7:507-550.
- Rothschild, M., Reichstein, T., von Euw, J., Alpin, R., and Harman, R.R.M. 1970. Toxic Lepidoptera. *Toxicon* 8:293-299.
- Schoener, T.W. 1970. Theory of feeding strategies. *Ann. Rev. Ecol. Syst.* 2:369-404.
- Schroeder, L.A. 1976. Energy, matter and nitrogen utilization by the larvae of the monarch butterfly Danaus plexippus. *Oikos* 27:259-264.
- Schroeder, L.A. 1977. Energy, matter and nitrogen utilization by larvae of the milkweed tiger moth Euchaetias egle. *Oikos* 28:27-31.
- Scriber, J.M. 1979. The effects of sequentially switching foodplants upon biomass and nitrogen utilization by polyphagous and stenophagous Papilio larvae. *Ent. exp. appl.* 25:203-215.
- Scriber, J.M., and Feeny, P.P. 1979. Growth of herbivorous caterpillars in relation to feeding specialization and to the growth form of their food plants. *Ecology* 60:829-850.
- Scriber, J.M., and Slansky, F., Jr. 1981. The nutritional ecology of immature insects. *Ann. Rev. Entomol.* 26:183-211.
- Scudder, G.G.E., and Duffey, S.S. 1972. Cardiac glycosides in the Lygaeinae (Hemiptera: Lygaeidae). *Can. J. Zool.* 50:35-42.
- Seiber, J.N., Lee, M.S., and Benson, J.M. In press. Cardiac glycosides (cardenolides) in species of Asclepias (Asclepiadaceae), In: A.T. Tu and R.F. Keeler (eds), *Encyclopedic handbook of natural toxins*. Vol. I. Plant and fungal toxins. Marcel Dekker, New York.
- Seiber, J.N., Tuskes, P.M., Brower, L.P., and Nelson, C.N. 1980. Pharmacodynamics of some individual milkweed cardenolides fed to larvae of the monarch butterfly (Danaus plexippus L.). *J. Chem. Ecol.* 6:321-339.
- Singer, M.C. 1971. Evolution of food-plant preference in the butterfly Euphydryas editha. *Evolution* 25:383-389.
- Smiley, J. 1978. Plant chemistry and the evolution of host specificity: new evidence from Heliconius and Passiflora. *Science* 201:745-747.

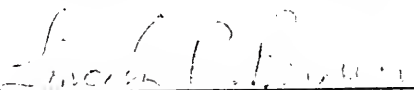
- Smith, B.D. 1966. Effect of the plant alkaloid sparteine on the distribution of the aphid Acyrtosiphon spartii. *Nature* 212:213-214.
- Smith, D.A.S. 1978. The effects of cardiac glycoside storage on growth rate and adult size in the butterfly Danaus chrysippus (L.). *Experientia* 34:845-846.
- Sokal, R.R., and Rohlf, F.J. 1981. *Biometry*. 2nd edit. W.H. Freeman Co., San Francisco.
- Takhtajan, A. 1969. *Flowering plants. Origin and dispersal*. Oliver and Boyd, Edinburgh.
- Thompson, J.N. 1982. *Interaction and coevolution*. Wiley Interscience, New York.
- Tietz, H.M. 1972. An index to the described life histories, early stages, and hosts of the Macrolepidoptera of the continental United States and Canada. A.C. Allyn Mus. Entomol., Sarasota, Fla.
- Tunnickliff, E.A., and Cory, V.L. 1930. Broad-leafed milkweed (Asclepias latifolia) poisonous for sheep and goats. *J. Am. Vet. Med. Assn.* 77:165-168.
- Urquhart, F.A. 1960. *The monarch butterfly*. Univ. of Toronto Press, Toronto.
- Urquhart, F.A., and Urquhart, N.R. 1976. A study of the peninsular Florida populations of the monarch butterfly (Danaus p. plexippus; Danaidae). *J. Lepidop. Soc.* 30:73-87.
- Urquhart, F.A., and Urquhart, N.R. 1977. The overwintering site of the eastern population of the monarch butterfly (Danaus plexippus; Danaidae) in southern Mexico. *J. Lepidop. Soc.* 30:153-158.
- van Emden, H.F. 1972. Aphids as phytochemists. In: J.B. Harborne (ed), *Phytochemical ecology*. Academic Press, New York.
- Vaughan, F.A. 1979. Effect of gross cardiac glycoside content of seeds of common milkweed Asclepias syriaca, on cardiac glycoside uptake by the milkweed bug Oncopeltus fasciatus. *J. Chem. Ecol.* 5:89-100.
- Vaughan, G.L., and Jungreis, A.M. 1977. Insensitivity of lepidopteran tissues to ouabain: physiological mechanisms for protection from cardiac glycosides. *J. Insect Physiol.* 23:585-589.

- von Euw, J., Fishelson, L., Parsons, J.A., Reichstein, T., and Rothschild, M. 1967. Cardenolides (heart poisons) in a grasshopper feeding on milkweeds. *Nature* 214:35-39.
- Waldbauer, G.P. 1968. The consumption and utilization of food by insects. *Adv. Insect Physiol.* 5:229-288.
- Walford, P., and Brower, L.P. In prep. Lipids in the migration cycle of the monarch butterfly, Danaus plexippus.
- Whittaker, R.H., and Feeny, P.P. 1971. Allelochemicals: chemical interactions between species. *Science* 171:757-770.
- Wilbur, H.M. 1976. Life history evolution in seven milkweeds of the genus Asclepias. *J. Ecol.* 64:223-240.
- Williams, K.S., and Gilbert, L.E. 1981. Insects as selective agents on plant vegetative morphology: egg mimicry reduces egg laying by butterflies. *Science* 212:467-469.
- Willis, J.C. 1931. A dictionary of the flowering plants and ferns. 6th edit. Cambridge Univ. Press, Cambridge.
- Wilson, E.O., and W.H. Bossert. 1971. A primer of population biology. Sinauer Assoc., Sunderland, Ma.
- Woodson, R.E. 1954. The North American species of Asclepias L. *Ann. Missouri Bot. Gard.* 41:1-261.
- Young, A.M. 1982. An evolutionary-ecological model of the evolution of migratory behavior in the Monarch Butterfly, and its absence in the Queen Butterfly. *Acta Biotheor.* 31:219-237.
- Zucker, W.V. 1983. Tannins: does structure determine function? An ecological perspective. *Am. Nat.* 121:335-365.

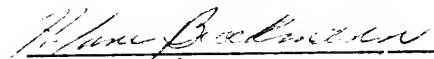
BIOGRAPHICAL SKETCH

James A. Cohen was born in New York in 1952 and grew up on Long Island. In 1974 he received his B.A. in psychology from Washington University (St. Louis, Mo.), where he graduated summa cum laude. His M.S. in Zoology was awarded three years later by the State University of New York, College of Environmental Science and Forestry (Syracuse, N.Y.). After a year's appointment as Scientific Consultant to the World Federation for the Protection of Animals (Zurich, Switzerland), he returned to do further graduate work in ecology and animal behavior at The Johns Hopkins University and the University of Florida. In addition to his work on the chemical interactions of plants and animals, Cohen's research has included studies of animal communication, motivational control, sexual selection, and behavioral ecology in a wide diversity of both invertebrate and vertebrate species, spanning three continents. Much of his spare time is devoted to worship of the pedal steel guitar.

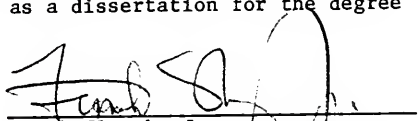
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Lincoln P. Brower, Chairman
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

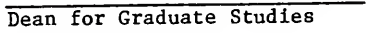

H. Jane Brockmann
Associate Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Frank Slansky Jr.
Assistant Professor of Entomology
and Nematology

This dissertation was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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